NTP REPORT ON CARCINOGENS BACKGROUND DOCUMENT for 6-NITROCHRYSENE

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TABLE OF CONTENTS

NTP Report on Carcinogens Listing for 6-Nitrochrysene

Carcinogenicity

6-Nitrochrysene is *reasonably anticipated to be a human carcinogen* based on evidence of carcinogenicity at multiple sites in multiple species of experimental animals (reviewed in IARC V.46, 1989).

In seven studies, when administered by intraperitoneal injection, 6-nitrochrysene caused lung tumors in male and/or female mice (Busby et al., 1985,1989; El-Bayoumy et al., 1992; Li et al., 1994; Fu et al., 1994; Imaida et al., 1992; Wislocki et al., 1986; cited by IARC V.46, 1989) and also induced liver tumors in female and/or male mice in three of these studies and malignant lymphoma in one. Dysplastic and/or adenomatous lesions of the colon were increased in male and female rats, and colon adenocarcinomas were increased in male rats receiving 6-nitrochrysene by intraperitoneal injection (Imaida et al., 1992). Mammary fibroadenoma, adenocarcinoma and spindle cell sarcomas were increased in female rats receiving 6-nitrochrysene by injection into the mammary gland (El-Bayoumy et al., 1993).

There are no adequate data available to evaluate the carcinogenicity of 6-nitrochrysene in humans.

Other Information Relating to Carcinogenesis or Possible Mechanisms of Carcinogenesis

6-Nitrochrysene induced skin tumors, mainly papillomas, in a dermal initiation-promotion study in which 6-nitrochrysene was used as the initiator, followed by promotion with a phorbol ester (El-Bayoumy et al., 1982; cited by IARC V.46, 1989). 6-Nitrochrysene caused lung and forestomach tumors when given by intraperitoneal injection to transgenic mice carrying a human hybrid c-Ha-*ras* gene (Ogawa et al., 1996). 6-Nitrochrysene is genotoxic in several assays in bacteria and mammalian cells, induces cell transformation in finite lifespan cells in vitro and metabolic pathways leading to mutagenic and clastogenic metabolites and DNA adducts have been described (IARC V.46, 1989). Evidence for 6-nitrochrysene-DNA adducts in tumor target tissue supports further the possibility that tumors induced by this chemical are at least in part a result of chemical-induced DNA damage.

No data are available that would suggest that the mechanisms thought to account for tumor induction by 6-nitrochrysene in experimental animals would not also operate in humans.

Listing Criteria from the Report on Carcinogens, Eighth Edition

Known To Be A Human Carcinogen:

There is sufficient evidence of carcinogenicity from studies in humans, which indicates a causal relationship between exposure to the agent, substance or mixture and human cancer.

Reasonably Anticipated To Be A Human Carcinogen:

There is limited evidence of carcinogenicity from studies in humans, which indicates that causal interpretation is credible but that alternative explanations, such as chance, bias, or confounding factors, could not adequately be excluded; or

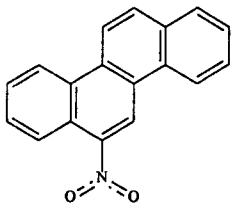
There is sufficient evidence of carcinogenicity from studies in experimental animals which indicates there is an increased incidence of malignant and/or a combination of malignant and benign tumors: (1) in multiple species or at multiple tissue sites, or (2) by multiple routes of exposure, or (3) to an unusual degree with regard to incidence, site or type of tumor, or age at onset; or

There is less than sufficient evidence of carcinogenicity in humans or laboratory animals; however, the agent, substance or mixture belongs to a well-defined, structurally related class of substances whose members are listed in a previous Report on Carcinogens as either a known to be human carcinogen or reasonably anticipated to be a human carcinogen, or there is convincing relevant information that the agent acts through mechanisms indicating it would likely cause cancer in humans.

Conclusions regarding carcinogenicity in humans or experimental animals are based on scientific judgement, with consideration given to all relevant information. Relevant information includes, but is not limited to dose response, route of exposure, chemical structure, metabolism, pharmacokinetics, sensitive sub populations, genetic effects, or other data relating to mechanism of action or factors that may be unique to a given substance. For example, there may be substances for which there is evidence of carcinogenicity in laboratory animals but there are compelling data indicating that the agent acts through mechanisms which do not operate in humans and would therefore not reasonably be anticipated to cause cancer in humans.

1.0 INTRODUCTION

6-Nitrochrysene
[7496-02-8]



1.1 Chemical Identification

6-Nitrochrysene ($C_{18}H_{11}NO_2$, mol. wt. = 273.29) is also called:

Chrysene, 6-nitro-
6-NC

1.2 Physical-Chemical Properties

Property	Information	Reference
Color	Chrome-red, Orange-yellow	Prager and Jacobson (1922); Boit (1965); both cited by IARC, (1989)
Physical State	Thick prismatic crystals, needle crystals	Prager and Jacobson (1922); Boit (1965); both cited by IARC, (1989)
Melting Point, °C	209	Boit (1965, cited by IARC, 1989)
Boiling Point, °C	Sublimes without decomposition	Prager and Jacobson (1922; cited by IARC, 1989)
Solubility:		
Organic Solvents	Slightly soluble in cold ethanol, diethyl ether, and carbon disulfide; Somewhat soluble in benzene and acetic acid; Soluble in hot nitrobenzene	Prager and Jacobson (1922; cited by IARC, 1989)

1.3 Identification of Structural Analogues and Metabolites

Structural analogues and metabolites discussed in this report included the following:

6-Aminochrysene (6-AC, $C_{18}H_{13}N$, MW = 243.31)
Chrysene-5,6-quinone ($C_{18}H_{11}NO_2$, MW = 273.29)
trans-1,2-Dihydrodiol-6-aminochrysene (1,2-DHD-6-AC, $C_{18}H_{13}NO_2$, MW = 277.32)
1,2,-Dihydrodiol-6-aminochrysene-3,4-epoxide (1,2-DHD-6-AC-3,4-epoxide, $C_{18}H_{11}NO_3$, MW = 289.29)
trans-1,2-Dihydrodiol-6-nitrochrysene (6-NC-1,2-dihydrodiol, 1,2-DHD-6-NC, $C_{18}H_{11}NO_4$, MW = 305.29)
trans-9,10-Dihydrodiol-6-nitrochrysene (9,10-DHD-6-NC, $C_{18}H_{11}NO_4$, MW = 305.29)
N-Formyl-6-aminochrysene (6-FAC, $C_{19}H_{13}NO$, MW = 271.32)
N-Hydroxy-6-aminochrysene (*N*-Hydroxy-6-AC, *N*-OH-6-AC, $C_{18}H_{13}NO$, MW = 259.31)
6-Nitrosochrysene (6-NOC, $C_{18}H_{11}NO$, MW = 257.29)

Physical-chemical properties could not be found for the above compounds. Structures for at least some of these analogues may be found in Figure 6-1.

1.4 Report Organization

The rest of this report is organized into six additional sections (2.0 Exposure Assessment, 3.0 Human Studies, 4.0 Mammalian Carcinogenicity, 5.0 Genotoxicity, 6.0 Other Relevant Studies, and 7.0 References) and two appendixes. Appendix A describes the literature search in online databases, and Appendix B provides explanatory information for Figure 5-1.

2.0 EXPOSURE ASSESSMENT

2.1 Use

6-Nitrochrysene (6-NC) is used as an internal standard in the chemical analysis of nitroarenes. It is available for research purposes at ≥98% purity and is also available at a certified purity of 98.9% as a reference material. No evidence has been found that 6-NC has been used commercially (IARC, 1989).

2.2 Production

One U.S. company produces 6-NC (SRI, 1992). No data on imports or exports of 6-NC were available. Chem Sources (1996) identified three U.S. suppliers including the NCI Chemical Carcinogen Reference Standard Repository.

2.3 Human Exposure

The primary route of potential human exposure to 6-NC is inhalation. Low concentrations of 6-NC have been found in ambient airborne particulates. Prior to 1980, some carbon black samples known to be used in photocopy machines were found to contain considerable quantities of nitropyrenes (IARC, 1989).

In Houston, Texas, the concentration of 6-NC in vapor plus particulate phase in ambient air was 2.3 pg/m^3 when measured over a 12-month period by GC-MS. The compound was found primarily in the vapor phase; however, the fraction in this phase varied substantially over 12

months. The authors stated that the phase distribution was related to the molecular size of the compound and weakly related to the ambient air temperature (Wilson et al., 1995).

6-NC was not listed in the National Occupational Exposure Survey (NIOSH, 1990) or the National Occupational Hazard Survey (NIOSH, 1977) conducted by NIOSH.

2.4 Regulations

OSHA regulates 6-NC under the Hazard Communication Standard and as a chemical hazard in laboratories.

REGULATIONS

	Regulatory Action	Effect of Regulation/Other Comments
OSHA	29 CFR 1910.1200. Promulgated 2/15/89. OSH Act: Hazard Communication Standard.	Requires chemical manufacturers and importers and all employers to assess chemical hazards and to provide information to employees. Hazard Communication program to include labels, material safety data sheets, and worker training.
	29 CFR 1910.1450. Promulgated 1/31/90. Amended 55 FR 12111, 3/30/90. OSH Act: Final rule for occupational exposure to hazardous chemicals in laboratories.	As a select carcinogen (IARC Group 2B), 6-nitrochrysene is included as a chemical hazard in laboratories. Employers are required to provide employee information and training and a Chemical Hygiene Plan.

3.0 HUMAN STUDIES

No studies were found that evaluated the carcinogenicity of 6-nitrochrysene in humans.

4.0 MAMMALIAN CARCINOGENICITY

Full details of mammalian carcinogenicity studies of 6-NC are presented in Table 4-1.

4.1 Mice

The incidence of lung tumors was significantly increased in newborn male and female Swiss-Webster BLU:Ha mice treated with a 7.7 μg (0.028 μmol) total dose of 6-NC (adenoma and adenocarcinoma; Busby et al., 1989), in newborn Hsd:ICR male and female mice treated with a 0.10 μmol total dose (tumor type not specified; El-Bayoumy et al., 1992), in newborn male CD-1 mice treated with a 0.25 μmol total dose (females were not included in the study) (adenoma and adenocarcinoma; Li et al., 1994), in newborn male and female Swiss-Webster BLU:Ha mice treated with a 38.5 μg (0.14 μmol) or 189 μg (0.69 μmol) total dose (adenoma and adenocarcinoma; Busby et al., 1985), in newborn male and female CrJ:CD-1 (ICR) mice treated

with a 1.4 μmol dose (adenoma; Imaida et al., 1992), and in newborn male and female CD-1 mice treated with a 0.7 or 2.8 μmol total dose (Wislocki et al., 1986; cited by IARC, 1989). In all studies, animals were treated over a 15-day period. More specific dosing schedules for these studies are provided in Table 4-1.

In the Imaida et al. (1992) study, no colon or liver tumors were detected in the 6-NC-treated mice. However, the incidence of liver tumors was significantly increased in newborn male, but not female, Hsd:ICR mice treated with a total dose of 0.10 μmol (1/7 of total dose administered at birth, 2/7 on day 8, 4/7 on day 15) (tumor type not specified) (El-Bayoumy et al., 1992) and in newborn male and female CD-1 mice treated with a total dose of 0.7 or 2.8 μmol (1/7 of total dose administered at birth, 2/7 on day 8, 4/7 on day 15) (adenoma and carcinoma) (Wislocki et al., 1986; cited by IARC, 1989).

In a study of the effect of caloric restriction on tumor induction by 6-NC, Fu et al. (1994) reported that a significantly greater number of newborn male B6C3F1 mice treated with a 0.4 μmol total dose of 6-NC (treatment 1: 1/7 of total dose administered at birth, 2/7 on day 8, 4/7 on day 15; treatment 2: 3/7 of total dose administered on day 8, 4/7 on day 15) and fed *ad libitum* developed liver adenomas and carcinomas than did vehicle controls fed *ad libitum*. When other groups of these mice were similarly treated, but were fed a calorically restricted diet, there was not a significant increase in the incidence of liver adenomas or carcinomas as compared to vehicle controls fed a calorically restricted diet.

In the same study, Fu et al. (1994) also reported that the incidence of lung tumors was significantly increased in newborn male B6C3F1 mice treated with a 0.4 μmol total dose of 6-NC (treatment 1) and fed *ad libitum* as compared to vehicle controls fed *ad libitum*. Lung tumor incidence in treated mice fed a calorically restricted diet was significantly lower than the incidence in treated mice fed *ad libitum*. The incidence of lung tumors in treated animals fed *ad libitum* was significantly greater than that in vehicle controls fed *ad libitum*, but the incidence of lung tumors in treated animals fed a calorically restricted diet was not significantly different from vehicle controls fed a calorically restricted diet.

In a study of the effect of the human hybrid c-Ha-*ras* gene on tumor induction, Ogawa et al. (1996) reported that 9-week-old male and female c57BL/6xBALB/c transgenic mice containing the human hybrid c-Ha-*ras* gene when treated with 2.2 μmol 6-NC biweekly, 3 times/wk for 3 weeks and sacrificed at the end of week 25 had a significantly higher incidence of lung tumors (males: adenoma, but not carcinoma; females: carcinoma, but not adenoma) and forestomach tumors (males: squamous cell carcinoma, but not papilloma; females: papillomas, but not squamous cell carcinoma) than did non-transgenic mice of the same strain.

4.2 Rats

4.2.1 Intraperitoneal Injection

The incidence of colon dysplasia and/or adenoma was significantly increased in newborn male and female Crj:CD (SD) rats treated over a 29-day period with a 14.8 μmol total dose of 6-NC as compared to vehicle controls. The incidence of colon adenocarcinoma was significantly increased in 6-NC-treated males, but not in 6-NC-treated females. Lesions were not observed in the lungs and livers of these rats (Imaida et al., 1992).

4.2.2 Mammary Injection

The incidences of mammary fibroadenoma, adenocarcinoma, and spindle cell sarcoma were significantly increased in 30-day-old female CD rats treated with a 12.3 μmol total dose of 6-NC (injected once into mammary tissue below each of 3 left thoracic nipples and once into inguinal nipples [side not specified]) as compared to vehicle controls (El-Bayoumy et al., 1993). The incidence of mammary tumors on the left side of 6-NC-treated rats was significantly greater than the incidence of mammary tumors on the right side of 6-NC-treated rat.

Table 4-1. Mammalian Carcinogenicity of 6-Nitrochrysene

Age, Strain, Species	No. and Sex Exposed	Controls	Chemical Form and Purity	Dose	Duration of Exposure	Results/Comments	Reference
Mice - *Dermal Application*							
50- to 55-day-old CD-1 Charles River mouse	20F	20F (acetone alone)	6-NC, > 99% pure	0.1 mg (0.4 µmol) in 0.1 mL acetone, applied on shaved dorsal skin every other day	20 days	Ten days after the completion of 6-NC treatment, all mice received applications of 2.5 µg *O*-tetradecanoylphorbol 13-acetate in 0.1 mL acetone 3 times per week for 25 weeks. Mice were evaluated for neoplasms at the end of this 25 week period. The statistical test used to analyze tumor incidence was not specified by IARC. **Skin:** Positive (for tumorigenesis) Skin tumor incidence (mainly papillomas; 2.1 tumors/animal) was significantly increased in 6-NC-treated mice (12/20 vs. 1/20 controls).	El-Bayoumy et al. (1982; cited by IARC, 1989)
Mice *Intraperitoneal Injection*							
newborn Swiss-Webster BLU:Ha mouse	effective number: 26M, 22F	effective number: 91M, 101F (DMSO alone	6-NC, > 99.9% pure	7.7 µg (0.028 µmol) total dose in DMSO, injected i.p. 1/7 of total dose (1.1 µg; 0.004µmol) administered at birth, 2/7 (2.2 µg; 0.008 µmol) on day 8, 4/7 (4.4 µg; 0.016 µmol) on day 15	15 days	Mice were killed when they were 26 weeks old. Lungs and any abnormal tissue were examined histologically. The significance of tumor incidence was calculated using the method of Peto et al. (1980; cited by Busby et al., 1989). The significance of tumor multiplicity was calculated with the Student's t-test. **Lungs:** Positive (for carcinogenesis) The incidence of lung tumors (adenoma and adenocarcinoma) was significantly increased in 6-NC-treated males and females (combined into 1 group; 32/48 vs. 20/192 controls; $p < 0.0001$). The multiplicity of lung tumors (adenoma and adenocarcinoma) was significantly increased in 6-NC-treated males and females (combined into 1 group; 2.77 ± 0.61 tumors/animal vs. 0.11 ± 0.03 tumors/control; $p < 0.001$).	Busby et al. (1989)

Table 4-1. Mammalian Carcinogenicity of 6-Nitrochrysene (Continued)

Age, Strain and Species	Number and Sex Exposed	Controls	Chemical Form and Purity	Dose	Duration of Exposure	Results/Comments	Reference
Mice *Intraperitoneal Injection (Continued)*							
Newborn Hsd:ICR mouse	38M, 24F	25M, 27F (DMSO alone)	6-NC, purity not specified	0.10 µmol total dose in DMSO, injected i.p. 1/7 of total dose (0.014 µmol) administered at birth, 2/7 (0.029 µmol) on day 8, 4/7 (0.057 µmol) on day 15	15 days	Only mice that survived more than 26 weeks were included in the study. Surviving mice were killed 30 weeks after the last injection. Lungs and liver were examined for presence of tumors, but histologic diagnosis of tumor type was not performed. Statistical analysis of tumor incidence was performed using the χ^2 test. Statistical analysis of tumor multiplicity was performed using the Student's *t*-test. **Liver:** Positive (for tumorigenesis; males only) Males treated with 6-NC had a significantly increased incidence (24/38 vs. 2/25 controls; $p < 0.001$) and multiplicity (7.2 ± 8.4 tumors/animal vs. 0.08 ± 0.28 tumors/control; $p < 0.001$) of liver tumors (type not specified). None of the 6-NC-treated or control females developed liver tumors. **Lungs:** Positive (for tumorigenesis; males and females) The incidence of lung tumors (type not specified) was significantly increased in 6-NC-treated males (38/38 vs. 3/25 controls; $p < 0.001$) and females (23/24 vs. 1/27 controls; $p < 0.001$). The multiplicity of lung tumors (type not specified) was also significantly increased in 6-NC-treated males (8.2 ± 5.4 tumors/animal vs. 0.12 ± 0.33 tumors/control; $p < 0.001$) and females (17.7 ± 11.3 tumors/animal vs. 0.07 ± 0.38 tumors/control; $p < 0.001$).	El-Bayoumy et al. (1992)

Table 4-1. Mammalian Carcinogenicity of 6-Nitrochrysene (Continued)

Age, Strain and Species	Number and Sex Exposed	Controls	Chemical Form and Purity	Dose	Duration of Exposure	Results/Comments	Reference
Mice *Intraperitoneal Injection (Continued)*							
newborn B6C3F1 mouse	19M (treatment 1 ; fed *ad libitum* [AL]) 21M (treatment 2 ; fed AL) 23M (treatment 1; caloric restriction) 22M (treatment 2; caloric restriction)	18M (DMSO alone; fed AL) 19M (DMSO alone; caloric restriction)	6-NC, > 99% pure	*treatment 1*: 0.400 µmol total dose in DMSO, injected i.p. 1/7 of total dose (0.057 µmol) administered at birth, 2/7 (0.114 µmol) on day 8, 4/7 (0.229 µmol) on day 15 *treatment 2*: 0.400 µmol total dose in DMSO, injected i.p. 3/7 of total dose (0.171 µmol) administered at 8 days of age, 4/7 (0.229 µmol) at 15 days of age	15 days	At 14 weeks of age, calorically restricted mice were fed 90% of the calories consumed by the AL mice, at 15 weeks of age, they were fed 75% of the calories consumed by the AL mice, and at 16 weeks of age until death, they were fed 60% of the calories consumed by the AL mice. Animals were killed at 12 months of age. The liver and lungs were examined histologically. Statistical analyses were performed using Fisher's Exact test for heterogeneity. **Liver:** Positive (for tumorigenesis; only in animals fed AL) All 6-NC-treated mice fed AL developed adenomas (treatment 1, 19/19; treatment 2, 21/21 vs. 2/18 controls fed AL; $p < 0.00001$) . All AL mice that received treatment 2 (21/21) and 74% (14/19) of AL mice that received treatment 1 developed carcinomas (vs. 0/18 controls fed AL; $p < 0.00001$). None of the mice that received treatment 2 or DMSO alone and were fed a calorically restricted diet developed adenomas or carcinomas. Mice that received treatment 1 and were fed a calorically restricted diet developed adenomas and carcinomas, but these incidences were not significantly different from those in calorically restricted vehicle controls. 6-NC-treated mice fed AL had multiple (> 5-8) tumors per liver section examined vs. 1 tumor/section in controls fed AL. 6-NC-treated mice that were calorically restricted had fewer tumors per section (treatment 1: 1.8/section; treatment 2: 0/section vs. no tumors in calorically restricted controls). **Lungs:** Positive (for tumorigenesis; only in animals fed AL) Tumor incidence in mice that received treatment 1 and were fed AL was significantly greater than tumor incidence in vehicle controls fed AL (11/19 vs. 1/18 controls; $p < 0.001$). Lung tumors were not detected in any of the 6-NC-treated mice that were fed a calorically restricted diet.	Fu et al. (1994a)

Table 4-1. Mammalian Carcinogenicity of 6-Nitrochrysene (Continued)

Age, Strain and Species	Number and Sex Exposed	Controls	Chemical Form and Purity	Dose	Duration of Exposure	Results/Comments	Reference
Mice *Intraperitoneal Injection (Continued)*							
newborn CD-1 mouse	21M	27M (DMSO alone) 14M (1.2 µmol B[*a*]P)	6-NC, purity not specified	0.250 µmol total dose in DMSO, injected i.p. 1/7 of total dose (0.0357 µmol) administered at birth, 2/7 (0.0714 µmol) on day 8, 4/7 (0.143 µmol) on day 15	15 days	Mice were killed after 11 months. Only lungs were examined histologically. No mention was made of statistical analysis of tumor incidence. **Lungs:** Positive (for tumorigenesis) All 6-NC-treated mice (21/21) developed adenomas vs. approximately 10% of controls (actual rate not given). Adenocarcinoma was detected in 5/21 6-NC-treated mice vs. none of the controls).	Li et al. (1994)
newborn Swiss-Webster BLU:Ha mouse	22M, 29F (low dose) 23M, 21F (high dose	22M, 15F (DMSO alone)	6-NC, purity not specified	38.5 µg (0.14 µmol) or 189 µg (0.69 µmol) total dose in DMSO, injected i.p. 1/7 of total dose (5.5 or 27 µg; 0.020 or 0.099 µmol) administered at birth, 2/7 (11 or 54 µg; 0.040 or 0.20 µmol) on day 8, 4/7 (22 or 108 µg; 0.080 or 0.39 µmol) on day 15	15 days	Mice were killed when they were 26 weeks old. Lungs and any abnormal tissues or organs were examined histologically. Statistical analyses of tumor incidence were performed using the method of Peto et al. (1980; cited by Busby et al., 1985), without correction for intercurrent mortality. Statistical analysis of tumor multiplicity was performed using the Student's *t*-test. **Lungs:** Positive (for tumorigenesis) The combined incidence (male and female) and multiplicity of lung tumors was significantly increased in mice treated with either dose of 6-NC (incidence: 51/51 [low-dose] and 44/44 [high-dose] vs. 4/37 controls; $p < 0.0001$; multiplicity: 20.84 ± 2.03 tumors/animal [low-dose] and 35.73 ± 4.57 tumors/animal [high-dose] vs. 0.14 ± 0.07 tumors/control; $p < 0.001$). Seventy percent of 6-NC-treated animals (low- and high-dose) developed malignant lung tumors (adenocarcinoma). **Lymphatic System:** Negative One low-dose male and 2 high-dose females developed lymphomas, whereas none of the controls did. However, this incidence was not statistically significant.	Busby et al. (1985)

Table 4-1. Mammalian Carcinogenicity of 6-Nitrochrysene (Continued)

Age, Strain and Species	Number and Sex Exposed	Controls	Chemical Form and Purity	Dose	Duration of Exposure	Results/Comments	Reference
Mice *Intraperitoneal Injection (Continued)*							
newborn CrJ:CD-1 (ICR) mouse (tumor induction study)	9M, 11F (tumor induction study)	22M, 26F (DMSO alone)	6-NC, >99.5% pure	*tumor induction study*: 1.4 µmol total dose in DMSO, injected i.p. 1/7 of total dose (0.2 µmol) administered at birth, 2/7 (0.4 µmol) on day 8, 4/7 (0.8 µmol) on day 15 *AHH activity assay*: 0.8 µmol/animal, single i.p. injection	15 days	Surviving mice were killed at 24 weeks of age. The major organs were examined grossly and microscopically. Statistical analyses of tumor incidence and multiplicity were performed using Fisher's exact probability test and Student's *t*-test, respectively. **Lungs:** Positive (for tumorigenesis) The incidence of lung adenoma was significantly increased in 6-NC-treated mice (9/9 males and 11/11 females vs. 0/22 male controls and 0/26 female controls; $p < 0.001$). 6-NC-treated males had a mean of 53.2 ± 18.1 adenomas/animal and 6-NC-treated females had a mean of 52.0 ± 26.3 adenomas/animal ($p < 0.001$). **Colon and Liver:** Negative No tumors were detected in colon or liver.	Imaida et al. (1992)

Table 4-1. Mammalian Carcinogenicity of 6-Nitrochrysene (Continued)

Age, Strain and Species	Number and Sex Exposed	Controls	Chemical Form and Purity	Dose	Duration of Exposure	Results/Comments	Reference
Mice *Intraperitoneal Injection (Continued)*							
newborn CD-1 mouse	"groups of 90 or 100" males and females	"groups of 90 or 100" males and females (DMSO alone) "groups of 90 or 100" males and females (DMSO alone; administered 10 wk after first vehicle control group) "groups of 90 or 100" males and females (positive control; 560 nmol B[*a*]P)	6-NC, > 99% pure	0.7 or 2.8 µmol total dose in 10, 20, and 40 µL DMSO, injected i.p. on day 1, 8, and 15 after birth Group administered 0.7 µmol total dose was begun 10 weeks after group administered 2.8 µmol total dose.	15 days	All surviving mice were killed after 1 year. The statistical test used to analyze tumor incidence was not specified by IARC. **Liver:** Positive (for tumorigenesis) Liver-cell tumors were detected in 3/9 high-dose males (3 carcinomas), in 3/11 high-dose females (2 adenomas, 1 carcinoma), in 25/33 low-dose males (1 adenoma, 24 carcinomas), and in 9/40 low-dose females (5 adenomas, 4 carcinomas). Two of 28 male controls (group 1), 5/45 male controls (group 2), and none of the female controls developed liver-cell tumors. Eighteen of 37 males treated with B[*a*]P developed liver tumors. **Lungs:** Positive (for tumorigenesis) Lung tumors were detected in 7/9 high-dose males, 9/11 high-dose females, 28/33 low-dose males (11 adenomas, 17 carcinomas), and 36/40 low-dose females (19 adenomas, 17 carcinomas). All lung tumor incidences in 6-NC-treated animals were significantly greater than those in controls (1/28 male controls in group 1, 4/45 males in control group 2, 0/31 females in control group 1, and 2/34 females in control group 2). Thirteen of 37 males and 13/27 females treated with B[*a*]P developed lung adenomas. **Lymphatic System:** Positive (for malignant lymphoma) The incidence (not given) of malignant lymphoma was significantly increased in 6-NC-treated mice. The incidence of malignant lymphoma was not significantly increased in B[*a*]P-treated animals.	Wislocki et al. (1986, Cited by IARC, 1989)

Table 4-1. Mammalian Carcinogenicity of 6-Nitrochrysene (Continued)

Age, Strain and Species	Number and Sex Exposed	Controls	Chemical Form and Purity	Dose	Duration of Exposure	Results/Comments	Reference
Mice *Intraperitoneal Injection (Continued)*							
9-wk-old non-transgenic C57BL/6xBA LB /c mice and transgenic C57BL/6xBA LB /c mice containing the human hybrid C-Ha-*ras* gene	number of 6-NC-exposed mice not specified, but 22M and 19F non-transgenic mice, and 19M and 15F transgenic mice were included in the study	number of DMSO controls not specified, but 22M and 19F non-transgenic mice, and 19M and 15F transgenic mice were included in the study	6-NC, purity not specified	2.2 μmol in DMSO, injected i.p., 3 times, at 2-week intervals	4 wk	Surviving mice were killed at the end of week 25. All organs were examined. Statistical analyses of tumor incidence and multiplicity were performed using Fisher's exact probability test and Student's *t*-test, respectively. **Lungs:** In 6-NC-treated mice, the incidence and multiplicity of adenoma (but not of carcinoma) in transgenic males (6/9; 1.0 ± 0.9 tumors/animal) was significantly greater ($p < 0.05$) than that in non-transgenic males (1/13; 0.1 ± 0.3 tumors/animal). The incidence and multiplicity of carcinoma (but not of adenoma) in transgenic females (4/7; 0.9 ± 0.9 tumors/animal) was significantly greater ($p < 0.05$) than that in non-transgenic females (0/10). Male and female vehicle controls (non-transgenic and transgenic) did not develop adenomas or carcinomas. Treated transgenic females were the only 6-NC-treated group that developed carcinoma (4/7; all were adenocarcinomas). Six of 9 6-NC-treated transgenic males, 3/7 6-NC-treated transgenic females, 1/13 6-NC-treated non-transgenic males, and 1/10 6-NC-treated non-transgenic females developed adenoma. Although the incidences of adenoma and carcinoma were greater in 6-NC-treated mice than in corresponding vehicle controls, a statistical analysis of significance was not performed. **Forestomach:** The incidence of squamous cell carcinoma (but not of papilloma) was significantly increased in transgenic 6-NC- treated males (6/9; $p < 0.05$) as compared to non-transgenic 6-NC-treated males (incidence not given). The incidence of papillomas (but not of squamous cell carcinoma) was significantly increased in transgenic 6-NC-treated females (5/7; $p < 0.05$) as compared to non-transgenic 6-NC-treated females (incidence not given). No mention was made of the incidence of forestomach tumors in vehicle controls.	Ogawa et al. (1996)

Table 4-1. Mammalian Carcinogenicity of 6-Nitrochrysene (Continued)

Age, Strain and Species	Number and Sex Exposed	Controls	Chemical Form and Purity	Dose	Duration of Exposure	Results/Comments	Reference
Rats - *Intraperitoneal Injection*							
newborn Crj:CD (SD) rat (tumor induction study)	31M, 32F	40M, 29F (DMSO alone)	6-NC, > 99.5% pure	*tumor induction study*: 14.8 µmol total dose in DMSO, injected i.p. in 5 doses, on days 1 (0.4 µmol), 8 (0.8 µmol), 15 (1.6 µmol), 22 (4.0 µmol), and 29 (8.0 µmol) on day 1 after birth.	29 days single dose	Surviving rats were killed when they were 32 weeks old. Lung, liver, kidneys, spleen, digestive tract, and other major organs were examined. Statistical analyses of tumor incidence and multiplicity were performed using Fisher's exact probability test and Student's *t*-test, respectively. **Colon:** Positive (for dysplasia and/or adenoma in males and females; for adenocarcinoma in males only) The incidence of dysplasia and/or adenoma was significantly increased in 6-NC-treated males (7/31 vs. 0/40 controls; $p < 0.01$) and females (14/32 vs. 0/29 controls; $p < 0.001$). The incidence of adenocarcinoma was significantly increased in 6-NC-treated males (5/31 vs. 0/40 controls; $p < 0.05$), but not in 6-NC-treated females (2/32 vs. 0/29 controls). **Other Organs:** Negative Lesions were not observed in other organs (including lung and liver).	Imaida et al. (1992)

Table 4-1. Mammalian Carcinogenicity of 6-Nitrochrysene (Continued)

Age, Strain and Species	Number and Sex Exposed	Controls	Chemical Form and Purity	Dose	Duration of Exposure	Results/Comments	Reference
Rats *Mammary Injections*							
30-day-old CD rat	30F	30F (DMSO alone	6-NC, > 99.8% pure	12.3 µmol total dose *day 1*: 100 µL of 6-NC solution (concentration not given) in DMSO injected directly into mammary tissue below each of 3 left thoracic nipples. Mammary tissue below right thoracic nipples injected with DMSO alone. *day 2*: 100 µL of 6-NC solution (concentration not given) injected directly into mammary tissue below inguinal nipples (side not specified)	single dose	Rats were killed 43 weeks after the second injection. All rats survived the duration of the experiment. All organs and the mammary glands were examined macroscopically. Mammary tumors were diagnosed histologically. Statistical analyses of tumor incidence were performed using the χ^2 method. Statistical analysis of tumor multiplicity was performed using the Student's *t*-test. **Mammary Gland:** Positive (for tumorigenesis) The incidences of fibroadenoma, adenocarcinoma, and spindle cell sarcoma were significantly increased in 6-NC-treated rats (24/30, 15/30, and 10/30, respectively, vs. 5/30, 0/30, and 0/30 controls, respectively; $p < 0.001$). The incidence of mammary tumors detected on the left side (25/30) was significantly greater than the number detected on the right side (9/30; $p < 0.001$). The multiplicity of left-side fibroadenomas, adenocarcinomas, and spindle cell sarcomas was significantly greater ($p < 0.001$) in treated rats than in controls and was significantly greater ($p < 0.001$) on the left side than on the right side.	El-Bayoumy et al. (1993)

5.0 GENOTOXICITY

Studies of the genotoxic effects of 6-NC and its metabolites are summarized in Table 5-1 and 5-2.

Summary: 6-NC was found to be positive in a number of prokaryotic and mammalian *in vitro* and *in vivo* test systems [see Genetic Activity Profile, Figure 5-1 (data limited to IARC, 1989)]. When tested *in vitro*, 6-NC was found to induce DNA damage in *Bacillus subtilis*; gene mutations in *Salmonella typhimurium* and repair-deficient Chinese hamster ovary (CHO) cells; DNA adducts in primary rat hepatocytes, human lymphoblastoid cells, CHO cells, and human bronchial tissue; and cell transformation in Syrian hamster embryo and rat tracheal epithelial cells. *In vivo*, it was positive for DNA adduct formation in various tissues of newborn mice and female rats. 6-NC was negative for induction of gene mutations in CHO cells and human lymphoblastoid cells as well as cell transformation in mouse C3H 10T1/2 and BALB/c3T3 cells.

Unless otherwise specified, rat liver S9 was the source of metabolic activation *in vitro*. Information for studies reviewed in IARC Vol. 46, 1989 was often limited to qualitative data with information on study design, doses tested, chemical purity, etc., generally not provided. For simplicity, multiple citations in IARC for the same genetic toxicity endpoint and test system are presented as a group rather than cited individually.

5.1 Noneukaryotic Systems

5.1.1 DNA Damage

As reported by Tokiwa et al. (1987; cited by IARC, 1989), 6-NC at 0.5 μg/disc (2 nmol/disc) inhibited the growth of a repair-deficient strain of *B. subtilis* in the absence of metabolic activation.

El-Bayoumy et al. (1989) evaluated DNA adduct formation in *S. typhimurium* strain TA100 after exposure to 4.6 μM 6-NC both in the presence and absence of a rat liver S9 metabolic activation system. In the presence of S9, most of the organic-soluble metabolites were derived from ring oxidation and a major DNA adduct was detected.

5.1.2 Gene Mutations

As reported by IARC (1989), 6-NC [LED = 0.5 μg/plate (2000 μmol/plate)] was found to give positive results for gene mutations in *S. typhimurium* strains TA98 and TA100 both in the presence and absence of S9 activation. El-Bayoumy et al. (1989) found that 6-NC displayed a dose-dependent increase in mutations in *S. typhimurium* strain TA100 from 4 to 40 nmol/plate in the presence and absence of S9.

5.2 Mammalian Systems *In Vitro*

5.2.1 DNA Damage

As presented by Delclos et al. (1987; cited by IARC, 1989), 6-NC at 2.7 μg/mL (10 μM) induced two major DNA adducts in primary rat liver hepatocytes. Delclos et al. (1987b) further reported that the DNA from exposed male Sprague-Dawley (SD) rat hepatocytes contained adducts derived from *N*-hydroxy-6-aminochrysene (*N*-hydroxy-6-AC), a product derived from the nitroreduction of 6-NC.

In a more recent study, Delclos et al. (1989) examined the influence of the induction of different drug-metabolizing enzymes on the activation pathway of 6-NC. They used hepatocytes taken from untreated SD rats or from SD male rats pretreated with phenobarbital, 3-methylcholanthrene (3-MCA), or Aroclor 1254. The hepatocytes were incubated with 10 μM 6-NC and adducts derived from *N*-hydroxy-6-AC were detected in hepatocytes from untreated and phenobarbital-pretreated rats.

Delclos et al. (1989) investigated 6-NC metabolism and DNA adduct formation in human bronchial tissue. Samples were obtained from 11 recently deceased patients and were incubated with 1 to 10 μM 6-NC. Metabolites formed via both ring oxidation and nitroreduction were detected. Most of the DNA adducts formed were derived from 1,2-DHD-6-AC, a product of ring oxidation followed by nitroreduction, and/or *N*-hydroxy-6-AC, a product of nitroreduction.

Delclos and Heflich (1992) found that incubation of repair-proficient CHO-K1 cells with 6-NC at 15 μg/mL (55 μM) produced adducts (as measured by ^{32}P-postlabeling) thought to be derived from either *N*-hydroxy-6-AC, a metabolite produced via nitroreduction of 6-NC, or an epoxide of 1,2-DHD-6-AC, which is produced via ring oxidation followed by nitroreduction of 6-NC. When the CHO-K1 cells were exposed to 6-NC in the presence of S9, one major adduct was formed from a metabolite that was thought to be derived from 6-NC via a combination of ring oxidation and nitroreduction.

Delclos and Heflich (1993) found that 2.5 to 20 μg/mL (9.1 to 73 μM) 6-NC for 5 hours induced a significant increase in DNA adducts in CHO K1-BH4 cells in the presence of S9. Using the ^{32}P-postlabeling technique, the authors also reported that a single major DNA adduct chromatographically identical to that induced by the metabolite 6-NC-1,2-dihydrodiol.

Morris et al. (1994) reported that ^{32}P-postlabeling of 6-NC at 2.5 to 20 μg/mL (9.1 to 73 μM) for 28 hours produced low levels of three DNA adducts in the human lymphoblastoid cell line AHH-1, which demonstrates cytochrome P-450 activity [LED = 5.0 μg/mL (18 μM)].

5.2.2 Gene Mutations

Delclos and Heflich (1992) found that 2.5 to 20 μg/mL (9.1 to 73 μM) 6-NC for 5 hours induced a significant increase in mutants in excision-repair-deficient UV5 cells in the presence [LED = 5.0 μg/mL (18 μM)] but not in the absence of S9 nor in repair-proficient CHO K1-BH4 cells with or without S9.

Morris et al. (1994) reported that 6-NC at 2.5 to 20 (HID) μg/mL (9.1 to 73 μM) for 28 hours did not significantly increase the *hprt* mutant frequency in human lymphoblastoid cell line AHH-1, which demonstrate cytochrome P-450 activity.

5.2.3 Cell Transformation

As compiled by IARC (1989), 6-NC was found to give positive results for morphological transformation in Syrian hamster embryo (SHE) cells [LED = 1.0 μg/mL (3.7 μM)] (DiPaolo et al. [1983] and Sala et al. [1987]; both cited by IARC, 1989) but not in BALB/c3T3 cells (HID = 40 μM) or C3H 10T1/2 cells (HID = 55 μM) (Sala et al., 1987; cited by IARC, 1989). All were tested only in the absence of metabolic activation. Sheu et al. (1994) also reported that 0.2 to 5.0 μg/mL (0.7 to 18 μM) 6-NC exposure for 48 hours failed to induce a positive response for morphological transformation in BALB/c3T3 clone A3-1-1 cells. Mitchell and Thomassen (1990) reported that 6-NC at 0.5 μg/mL (2 μM) for 24 hours induced a highly significant

increase in morphological transformations in tracheal epithelial cells obtained from male Fischer 344/N rats either induced with 50 mg/kg 3-methylcholanthrene (3-MCA) or uninduced (corn oil).

5.3 Mammalian Systems *In Vivo*

5.3.1 DNA Damage

Delclos et al. (1988) found that whole-body *in vivo* metabolism by newborn BLU:Ha (ICR) mice of 6-NC (dose levels not provided) resulted in the production of a major DNA adduct that was derived from the 6-NC metabolite 1,2-DHD-6-AC.

Chae et al. (1996) reported that 6-NC (total of 14.8 μmol/animal injected i.p. on days 1, 8, 15, 22, and 29) induced DNA adducts as measured by ^{32}P-postlabeling in colon, the target organ, as well as mammary gland fat pads, liver, and lungs of female CD rats. The lungs contained the highest level of DNA adducts 24 hours after the last administration.

5.4 Genotoxicity of 6-Nitrochrysene Metabolites

Studies of the genotoxic effects of 6-NC metabolites are summarized in Table 5-2.

5.4.1 6-Nitrosochrysene

Manjanatha et al. (1993) reported that 6-nitrosochrysene (6-NOC) induced DNA adducts in CHO K1-BH4 and excision-repair-deficient CHO-UV5 cells in the absence of metabolic activation as measured by ^{32}P-postlabeling. Cells were incubated for 5 hours with 0.5 μg 6-NOC/mL (2 μM) (K1 cells) or 0.2 μg 6-NOC/mL (0.8 μM) (UV5 cells). The adducts comprised 80% deoxyguanosine (dG) adducts and 20% deoxyadenosine (dA) adducts.

Using similar procedures and doses within the ranging from 0.25 to 2.5 μg/mL (1 to 9.7 μM), Delclos and Heflich (1992) found that 6-NOC induced two major and one minor adduct in CHO K1-BH4 cell DNA in the absence of S9.

Manjanatha et al. (1993) found that 0.5 (LED) to 1.75 μg/mL (2 to 6.4 μM) 6-NOC for 5 hours induced a significant mutagenic response at the *hprt* locus in CHO K1-BH4 cells as did 0.15 (LED) and 0.2 μg/mL (0.55 and 0.7 μM) in excision-repair-deficient UV5 cells. Following DNA sequencing, predominantly basepair substitutions at A:T were observed with 43% analyzed as AT-to-GC transversions.

Delclos and Heflich (1992) found that 0.25 (LED) to 2.5 μg/mL (1 to 9.7 μM) 6-NOC for 5 hours induced a significant mutagenic increase at the *hprt* locus in both excision-repair-deficient UV5 cells and repair-proficient CHO K1-BH4 cells in the absence but not the presence of S9.

5.4.2 6-Nitrochrysene-1,2-Dihydrodiol

Using the ^{32}P-postlabeling technique, Delclos and Heflich (1992) found that 6-nitrochrysene-1,2-dihydrodiol (6-NC-1,2-dihydrodiol) in the presence of S9 induced a single major DNA adduct chromatographically identical to that induced by 6-NC plus S9. The adduct was presumed to be derived from *N*-hydroxy-6-AC-1,2-dihydrodiol.

Delclos and Heflich (1992) also found that doses of 0.5 (LED) to 7.5 μg/mL (2 to 24 μM) 6-NC-1,2-dihydrodiol for 5 hours induced a significant increase in mutations at the *hprt* locus in excision-repair-deficient UV5 cells in the presence of S9 only but not in repair proficient CHO K1-BH4 cells (with or without S9).

5.4.3 6-Aminochrysene

Delclos and Heflich (1992) found a DNA adduct pattern identical to that derived from the *in vitro* reaction of *N*-hydroxy-6-AC with calf thymus DNA when CHO K1-BH4 cells were incubated in the presence of S9 with 2.5 μg/mL (10 μM) [and possibly other doses] 6-AC. The authors found that 6-AC in the presence of S9 was mutagenic at the *hprt* locus to both CHO K1-BH4 and CHO-UV5 cells [LED approximately 0.25 μg/mL (0.91 μM)]. The K1 cells were tested at concentrations from approximately 0.5 to 20 μg/mL (2 to 82 μM) and the UV5 cells from approximately 0.25 to 0.5 μg/mL (1 to 2 μM).

5.4.4 6-Aminochrysene-1,2-dihydrodiol

Continuing their testing of 6-NC metabolites, Delclos and Heflich (1992) found that 6-AC-1,2-dihydrodiol at 0.25 μg/mL (0.91 μM) induced a single DNA adduct in CHO K1-BH4 cells in the presence of S9. The authors presumed that the adduct was derived from 1,2-dihydroxy-3,4-epoxy-1,2,3,4-tetrahydro-6-AC. They also reported that 6-AC-1,2-dihydrodiol was mutagenic at the *hprt* locus in both CHO K1-BH4 and CHO-UV5 cells in the presence of S9 at concentrations of approximately 0.25 μg/mL (LED; 0.91 μM) to 0.5 μg/mL (UV5 cells, 2 μM) or 1.0 μg/mL (K1-BH4, 3.6 μM).

5.4.5 *N*-Hydroxy-6-aminochrysene

Delclos and Heflich (1992) reported, with few experimental details, that 5-*N*-hydroxy-6-AC formed calf thymus DNA adducts. The pattern of adducts in the autoradiogram resembled the adduct patterns from CHO cells treated with 6-AC and 6-NOC.

Table 5-1. Summary of 6-Nitrochrysene and 6-Nitrochrysene Metabolite Genotoxicity Studies

Test System	Biological Endpoint	S9 Metab. Activation	Purity	Doses Used	Endpoint Response	Comments	References
5.1 Noneukaryotic Systems							
5.1.1 DNA Damage							
Bacillus subtilis (DNA repair-deficient strain)	DNA damage (growth inhibition)		n.p.	0.5 μg/disk (2000 μmol/plate)	positive	None	Tokiwa et al. (1987; cited by IARC, 1989)
Salmonella typhimurium strain TA100	DNA adducts	+/-	n.p.	4.6 μM	positive/ negative	In the presence of S9, most of the organic-soluble metabolites were derived from ring oxidation and a major DNA adduct was detected.	El-Bayoumy et al. (1989)
5.1.2 Gene Mutations							
S. typhimurium strains TA100 and TA98	*His* gene mutations	+/-	n.p.	n.g.	positive/ positive	Salmonella results based on 5 papers, LED = 0.5 μg/plate (2000 μmol/plate)	5 papers cited by IARC, 1989
S. typhimurium strain TA100	*his* gene mutations	+/-	n.p.	4000 to 40,000 μmol/plate	positive/ positive	LED = 4000 μmol/plate	El-Bayoumy et al. (1989)
5.2 Mammalian Systems *In Vitro*							
5.2.1 DNA Damage							
primary rat hepatocytes	DNA adducts (^{32}P-postlabeling)	NA	n.p.	2.7 μg/mL (10 μM)	positive	6-NC induced two major DNA adducts derived from *N*-hydroxy-6-aminochrysene (*N*-hydroxy-6-AC), a product derived from the nitroreduction of 6-NC.	Delclos et al. (1987; cited by IARC, 1989); Delclos et al. (1987b)
Sprague-Dawley rat hepatocytes	DNA adducts (^{32}P-postlabeling)	NA	n.p.	10 μM	positive	Adducts derived from *N*-hydroxy-6-AC were detected in hepatocytes from untreated and phenobarbital-pretreated rats.	Delclos et al. (1989)

Table 5.1 Summary of 6-Nitrochrysene and 6-Nitrochrysene Metabolite Genotoxicity Studies (Continued)

Test System	Biological Endpoint	S9 Metab. Activation	Purity	Doses Used	Endpoint Response	Comments	References
5.2 Mammalian Systems *In Vitro* (Continued)							
5.2.1 DNA Damage							
human bronchial tissue	DNA adducts (^{32}P-postlabeling)	NA	n.p.	1 to 10 µM	positive	Levels of DNA binding varied in 11 specimens from 0.06 to 30.5 pmol [^{3}H]6-NC bound/mg DNA (2 adducts per 10^{8} nucleotides to 10 adducts per 10^{6} nucleotides).	Delclos et al. (1989)
CHO-K1 cells (repair-proficient)	DNA adducts (^{32}P-postlabeling)	+	n.p.	15 µg/mL (55 µM)	positive	6-NC produced adducts derived from either *N*-hydroxy-6-AC, a metabolite produced via nitroreduction of 6-NC, or an epoxide of 1,2-DHD-6-AC, produced via ring oxidation followed by nitroreduction of 6-NC.	Delclos and Heflich (1992)
Chinese hamster ovary K1-BH4 cells	DNA adducts (^{32}P-postlabeling)	+	n.p.	2.5 to 20 µg/mL [9.1 to 73 µM] for 5 h	positive	6-NC induced a single major adduct chromatographically identical to that induced by 6-NC-1,2-dihydrodiol.	Delclos and Heflich (1993)
human lymphoblastoid cell line AHH-1	DNA adducts (^{32}P-postlabeling)	NA	n.p.	2.5 to 20 µg/mL (9.1 to 73 µM) for 28 h	positive	6-NC produced low levels of three DNA adducts in AHH-1 cells, which demonstrates cytochrome P-450 activity. LED = 5.0 µg/mL (18 µM)	Morris et al. (1994)

Table 5.1 Summary of 6-Nitrochrysene and 6-Nitrochrysene Metabolite Genotoxicity Studies (Continued)

Test System	Biological Endpoint	S9 Metab. Activation	Purity	Doses Used	Endpoint Response	Comments	References
5.2.2 Gene Mutations							
Chinese hamster ovary K1-BH4 and excision repair deficient UV5 cells	*hprt* gene mutations	+/-	n.p.	2.5 to 20 μg/mL [9.1 to 73 μM] for 5 h	positive (UV5 only)/negative	6-NC significantly increased the mutant frequency only in UV5 cells in the presence of S9. LED = 5.0 μg/mL (18 μM)	Delclos and Heflich (1993)
human lymphoblastoid cell line AHH-1	*hrpt* gene mutations	NA	n.p.	2.5 to 20 μg/mL (9.1 to 73 μM) for 28 h	negative	6-NC did not significantly increase the hprt mutant frequency in AHH-1 cells, which retain native cytochrome P-450 activity. HID = 20 μg/mL (73 μM)	Morris et al. (1994)
5.2.3 Cell Transformation							
Syrian hamster embryo (SHE) cells	morphological transformation	NA	n.p.	1.0 to 20 μg/mL (3.7 to 73 μM)	positive	LED = 1.0 μg/mL (3.7 μM)	DiPaolo et al. (1983) & Sala et al. (1987; cited by IARC, 1989)
BALB/c3T3 and C3H 10T1/2 cells	morphological transformation	NA	n.p.	13 to 40 μM (BALB), 37 to 55 μM (C3H)	negative for both cell lines	HID = 40 μM (BALB), 55 μM (C3H)	Sala et al. 1987; cited by IARC, 1989)
BALB/3T3 cells clone A3-1-1	morphological transformation	NA	n.p.	0.2 to 5.0 μg/mL (0.7 to 18 μM) for 48 h	negative	HID = 5.0 μg/mL (18 μM)	Sheu et al. (1994)
male Fischer rat tracheal epithelial cells	Morphological transformation	NA	n.p.	0.5 μg/mL (2 μM) for 24 h	positive for both induced and uninduced rats	6-NC induced a highly significant increase in the transformation frequency in cells from both induced (50 mg 3-MCA/kg) and uninduced (corn oil) rats.	Mitchell and Thomassen (1990)

Table 5.1 Summary of 6-Nitrochrysene and 6-Nitrochrysene Metabolite Genotoxicity Studies (Continued)

Test System	Biological Endpoint	S9 Metab. Activation	Purity	Doses Used	Endpoint Response	Comments	References
5.3 Mammalian Systems *In Vivo*							
5.3.1 DNA Damage							
newborn BLU:Ha(ICR) mice	DNA adducts (^{32}P-postlabeling)	NA	n.p.	n.g.	positive	Whole body metabolism of 6-NC resulted in the production of a major DNA adduct that was derived from the metabolite 1,2-DHD-6-AC.	Delclos et al. (1988b)
female CD rats	DNA adducts (^{32}P-postlabeling)	NA	n.p.	14.8 μmol/rat i.p. on days 1, 8, 15, 22, and 29	positive	DNA adducts were found in the colon (target organ), mammary gland fat pads, liver, and lung (highest levels).	Chae et al. (1996)
5.4 6-Nitrochrysene Metabolites							
5.4.1 6-Nitrosochrysene (mol. wt. = 257.29)							
Chinese hamster ovary (CHO) K1-BH4 and excision repair deficient UV5 cells	DNA adducts (^{32}P-postlabeling)	-	n.p.	0.5 μg/mL (K1) [2 μM] or 0.2 μg/mL (UV5) [0.8 μM] for 5 h	positive for both cell lines	6-NOC induced 80% dG adducts and only 20% dA adducts.	Manjanatha et al. (1993)
CHO K1-BH4 cells	DNA adducts (^{32}P-postlabeling)	-	n.p.	Within range 0.25 to 2.5 μg/mL (1 to 10 μM) for 5 h	positive	Two major and one minor adduct spots were produced. Doses were not given explicitly	Delclos and Heflich (1992)

Table 5.1 Summary of 6-Nitrochrysene and 6-Nitrochrysene Metabolite Genotoxicity Studies (Continued)

Test System	Biological Endpoint	S9 Metab. Activation	Purity	Doses Used	Endpoint Response	Comments	References
CHO K1-BH4 and excision repair deficient UV5 cells	*hprt* gene mutations and DNA sequencing	-	n.p.	0.5 & 1.75 μg/mL (K1) [2 & 6.80 μM] or 0.15 & 0.2 μg/mL (UV5) [0.58 & 0.8 μM] for 5 h	positive for both cell lines	6-NOC induced predominantly basepair substitutions at A:T with 43% analyzed as AT to GC transversions. LED = 0.5 (K1) [2 μM] and 0.15 μg/mL (UV5) [0.58 μM]. Doses read from graph.	Manjanatha et al. (1993)
CHO K1-BH4 and excision repair deficient UV5 cells	*hprt* gene mutations	+/-	n.p.	0.25 to 2.5 μg/mL (1 to 10 μM) for 5 h	negative/positive for both cell lines	6-NOC significantly increased the mutant frequency in both cell lines in the absence of S9. LED = 0.25μg/mL (1.0 μM)	Delclos and Heflich (1992)
5.4.2 6-Nitrochrysene 1,2 dihydrodiol (mol. wt. = 307.30)							
CHO K1-BH4 cells	DNA adducts (^{32}P-postlabeling)	+	n.p.	Within range 0.5 to 7.5 μg/mL (2 to 24 μM) for 5 h	positive	The metabolite induced a single major adduct chromatographically identical to that induced by 6-NC.	Delclos and Heflich (1992)
CHO K1-BH4 and excision repair deficient UV5 cells	*hprt* gene mutations	+/-	n.p.	0.5 to 7.5 μg/mL (2 to 24 μM) for 5 h	positive (UV5 only)/negative	The metabolite significantly increased the mutant frequency only in UV5 cells in the presence of S9. LED = 0.5μg/mL (2 μM)	Delclos and Heflich (1992)
5.4.3 6-Aminochrysene (mol. wt. = 243.31)							
CHO K1-BH4 and excision repair deficient UV5 cells	*hprt* gene mutations	+/-	n.p.	0.25 to 20 μg/mL (1 to 82 μM) (from graphs)	positive/negative (both systems)	LED = 0.25 μg/mL (1.0 μM)	Delclos and Heflich (1992)

Table 5.1 Summary of 6-Nitrochrysene and 6-Nitrochrysene Metabolite Genotoxicity Studies (Continued)

Test System	Biological Endpoint	S9 Metab. Activation	Purity	Doses Used	Endpoint Response	Comments	References
5.4.4 6-Aminochrysene-1,2-dihydrodiol (mol. wt. = 275.31)							
CHO K1-BH4 cells	DNA adducts (^{32}P-postlabeling)	+	n.p.	0.25 μg/mL (0.91 μM) given in Figure 5	positive	Single adduct was distinct from those derived from *N*-hydroxy-6-AC. The authors presumed the adduct was derived from 1,2-dihydroxy-3,4-epoxy-1,2,3,4-tetrahydro-6-AC.	Delclos and Heflich (1992)
CHO K1-BH4 and excision repair deficient UV5 cells	*hprt* gene mutations	+/-	n.p.	0.25 to 1.0 μg/mL (0.91 to 3.6 μM) (from graph)	positive/ negative (both systems)	LED = 0.25 μg/mL (0.91 μM)	Delclos and Heflich (1992)
5.4.5 *N*-Hydroxy-6-aminochrysene (mol. wt. = 259.31)							
Calf thymus DNA	DNA adducts (^{32}P-postlabeling)	n.g.	n.p.	n.g.	positive	Adduct pattern resembled those from 6-AC and 6-nitrosochrysene.	Delclos and Heflich (1992)

Abbreviations: HID = highest ineffective dose; LED = lowest effective dose; NA = not applicable; n.g. = not given; n.p. = not provided.

Figure 5-1. Genetic Activity Profile of 6-Nitrochrysene (Data limited to IARC, 1989)

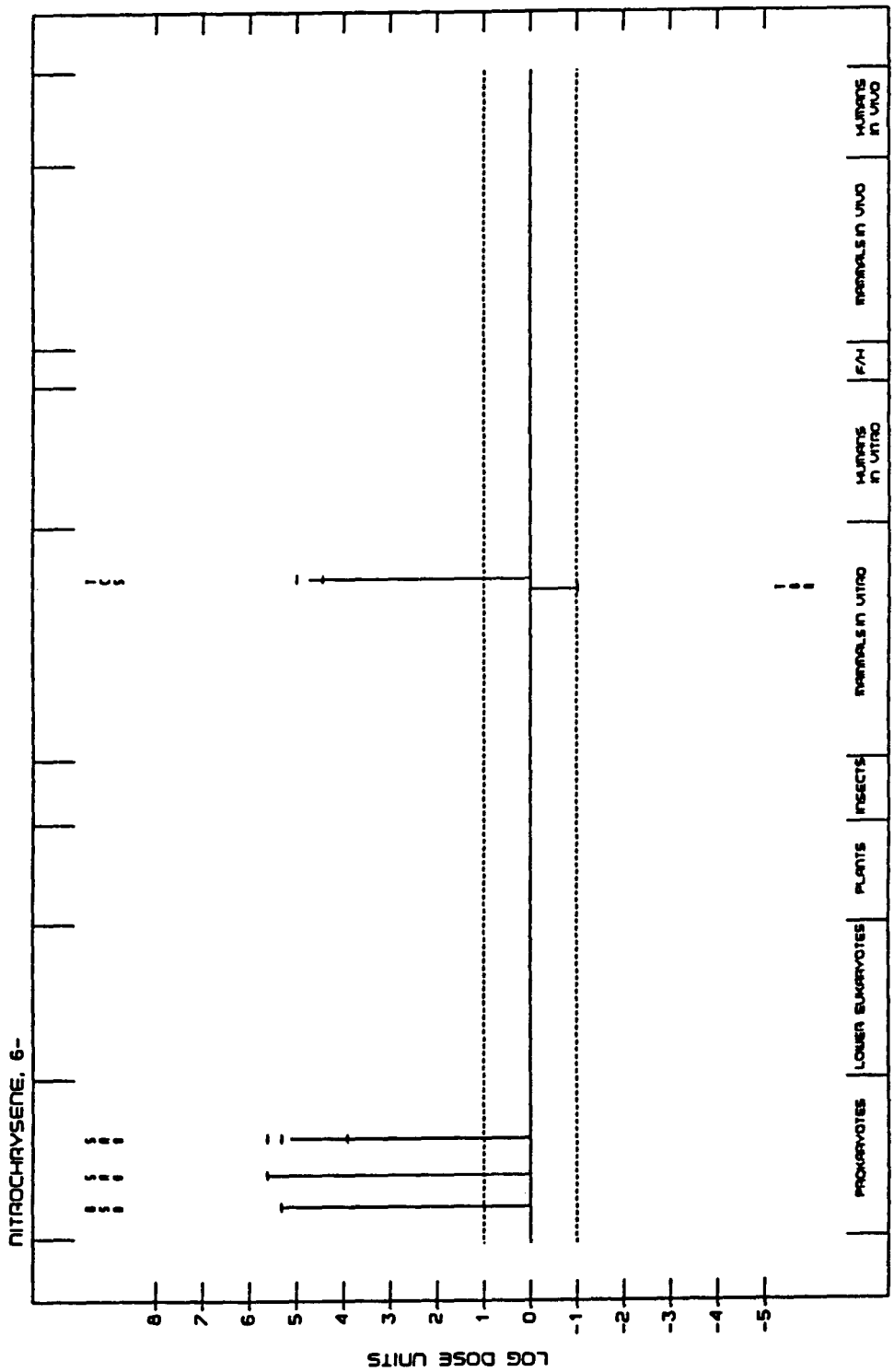
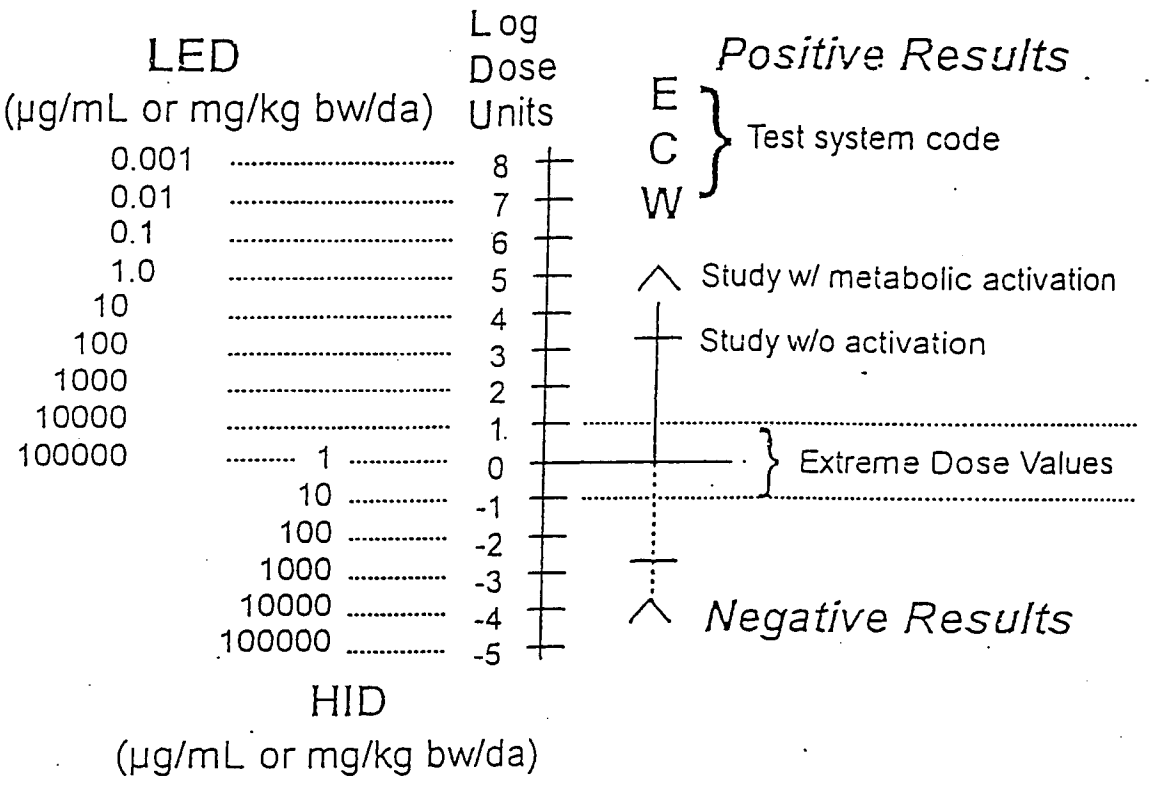


Figure 5-2. Schematic View of a Genetic Activity Profile (GAP)



A schematic view of a Genetic Activity Profile (GAP) representing four studies (two positive and two negative) for an example short-term test, ECW. Either the lowest effective dose (LED) or the highest ineffective dose (HID) is recorded from each study, and a simple mathematical transformation (as illustrated above) is used to convert LED or HID values into the logarithmic dose unit (LDU) values plotted in a GAP. For each test, the average of the LDUs of the majority call is plotted using a solid vertical bar drawn from the origin. A dashed vertical bar indicates studies that conflict with the majority call for the test. Note in cases where there are an equal number of positive and negative studies, as shown here, the overall call is determined positive. The GAP methodology and database have been reported previously (Garrett et al., 1984; Waters et al., 1988, 1991).

Garrett, N.E., H.F. Stack, M.R. Gross, and M.D. Waters. 1984. An analysis of the spectra of genetic activity produced by known or suspected human carcinogens. Mutat. Res. 143:89-111.

Waters, M.D., H.F. Stack, A.L. Brady, P.H.M. Lohman, L. Haroun, and H. Vainio. 1988. Use of computerized data listings and activity profiles of genetic and related effects in the review of 195 compounds. Mutat. Res. 205:295-312.

Waters, M.D., H.F. Stack, N.E. Garrett, and M.A. Jackson. 1991. The genetic activity profile database. Environ. Health Perspect. 96:41-45.

6.0 OTHER RELEVANT STUDIES

6.1 Absorption, Distribution, Metabolism, and Excretion

Summary: Summaries of the studies discussed in this subsection are presented in Table 6-1. Structures of many of the metabolites are shown in the metabolic pathways depicted in Figure 6-1 (Metabolic Activation of 6-Nitrochrysene).

6.1.1 Absorption, Distribution, and Excretion

Following i.p. administration of [^{3}H]6-NC (I; 9 μmol/rat), urine and feces were collected from Sprague-Dawley rats for 15 days. Within 24 hours, 2% of the dose was recovered in urine and 6% was excreted in feces. Fifteen days after administration of the dose, 6% of the radiolabel was excreted in urine and 10% in feces (Delclos et al., 1993 abstr.).

6.1.2 Metabolite and DNA Adduct Identification

Two major pathways have been implicated in the metabolic activation of 6-NC (for review see Li et al., 1994). The first of the two pathways involves the formation of *trans*-1,2-dihydro-1,2-dihydroxy-6-NC (III) via ring hydroxylation and subsequent nitroreduction to the intermediate *trans*-1,2-dihydro-1,2-dihydroxy-6-aminochrysene (V), and yields a single major DNA adduct (presently uncharacterized) (Delclos et al., 1987a, 1988a). The production of this unidentified adduct involves the formation of 1,2-dihydro-1,2-dihydroxy-6-aminochrysene-3,4-epoxide (VII) and results from reaction with deoxyguanosine (*hprt* gene of CHO cells at G:C basepairs) following *in vitro* incubations of (V) with CHO cells (Li et al., 1993).

As reported by Delclos et al. (1987b), sequential nitroreduction is involved in the second major activation pathway of 6-NC to 6-nitrosochrysene (II; 6-NOC) and *N*-hydroxy-6-aminochrysene (IV; N-OH-6-AC). Subsequently, (IV) is either further nitroreduced to 6-aminochrysene (VI; 6-AC) and ring hydroxylated to (V), or binds to DNA to form three major DNA adducts: 5-(deoxyguanosin-N^2-yl)-6-aminochrysene (5-[dG-N^2-yl]-6-AC; VIII), *N*-(deoxyguanosin-8-yl)-6-aminochrysene (*N*-[dG-8-yl]-6-AC; IX), or *N*-(deoxyinosin-8-yl)-6-aminochrysene (*N*-[DI-8-yl]-6-aminochrysene; XI). XI has been postulated by Djuric et al. (1987) to be derived from spontaneous oxidation of the deoxyadenosine adduct (*N*-[deoxyadenosine-8-yl]-6-aminochrysene; *N*-[DA-8-yl]-6-AC; X). It is not known if (XI) exists *in vivo* or was an artifact of the preparation of the modified nucleoside for structural determination by spectrometric analyses (Li et al., 1994).

Human hepatic and pulmonary microsomes metabolized 6-NC to potent mutagenic metabolites that were formed via ring hydroxylation or a combination of ring-hydroxylation and nitroreduction (III, 9,10-DHD-6-NC, V, VI, and chrysene-5,6-quinone). Using specific P-450 inhibitors and antibodies, and comparing catalytic activities known to be associated with a specific P-450 with the levels of each metabolite of 6-NC formed with the same microsomes, Chae et al. (1993) showed that P-450 IA2 and IIIA4 were well correlated with the rates of formation of (III) and (VI), respectively, in human liver.

Further metabolism of III was reported to require P-450 IIIA4. However, in human lung microsomes, P-450 IA1 was reported to play a major role in 6-NC metabolism to (III).

Using batch and semicontinuous culture systems to determine the ability of human, rat, and mouse intestinal microflora to metabolize 6-NC, Manning et al. (1988) reported that the rate of nitroreduction differed among the species studied. Human microflora metabolized 6-NC to (VI) to the greatest extent. In rat and mouse intestinal contents, the major organisms were species of *Eubacterium*, *Bacteroides*, and *Lactobacillus,* whereas human samples contained the above organisms as well as species of *Peptococcus*, *Bifidobacterium*, and *Actinomyces.* The greater diversity of intestinal microflora in humans might be responsible for the nitroreduction differences in the different test species reported by Manning et al. (1988).

Following i.p. administration of [^{3}H]6-NC to Sprague-Dawley rats, II, chrysene-5,6-dione, and (VI) were identified in feces 15 days after administration of the dose (Delclos et al., 1993 abstr.). One major metabolite was detected in urine but not identified. Both nitroreduction and a combination of ring oxidation and nitroreduction produced DNA adducts in rat livers (adducts not identified in abstract).

Different DNA adduct patterns were produced in the whole lung of CD-1 newborn mice following *in vivo* administration of (II) or (V). Adduct distributions ranged from 90% (~2.4 adducts/10^6 nucleotides) for the (XI) adduct derived from (IV) in the (II) treated group to 100% for a dG adduct (unidentified) formed from (V) (Li et al., 1994).

6.2 Pharmacokinetics

No pertinent information was found.

6.3 Modes of Action

Summary: 6-Nitrochrysene is genotoxic in several assays in bacteria and mammalian cells, and metabolic pathways leading to mutagenic and clastogenic metabolites, and DNA adducts, have been described (see Section 5.0).

Studies have shown that the activation of oncogenes plays a role in the carcinogenicity of 6-NC: K-*ras* mutations were detected in lung tumors in association with DNA adducts of 6-NC metabolites after treating mice with 6-NC for 15 days; in mice treated with 1,2-DHD-6-AC, all adenomas had a K-*ras* mutation involving a G:C basepair in codon 12 or 13 and the dG adduct accounted for >40% of the total identified adducts. However, in mice treated similarly with 6-NOC, the 1,2-DHD-6-AC-derived dG adduct accounted for 10% of the lung DNA adducts, and the N-hydroxy-6-AC-derived adduct accounted for 71% of the remaining adducts, which had the K-*ras* mutations at G:C.

DNA adducts of 6-NC metabolites have been found in cells of target organs when exposed *in vitro:* human bronchial cells and rat hepatocytes. 6-NC-derived adducts were mutagenic in the *hprt* gene of CHO cells; 82% of the identified mutations in the *hprt* gene occurred at adenine-thymidine base pairs.

6-NC induced skin tumors, mainly papillomas, in a dermal initiation-promotion study in which 6-NC was used as the initiator, followed by promotion with the phorbol ester. 6-NC caused lung and forestomach tumors following i.p. injection to transgenic mice carrying a human hybrid c-Ha-*ras* gene.

There is some evidence that the activation of oncogenes plays a role in the carcinogenicity of 6-NC. K-*ras* mutations were detected in lung tumors in association with DNA adducts of 6-NC metabolites after treating CD-1 mice with 6-NC of unspecified purity for 15 days (Li et al., 1994; the details of this study are presented in Table 4-1). In mice treated with

1,2-DHD-6-AC, the dG adduct accounted for >40% of the total identified adducts, and all adenomas had a K-*ras* mutation involving a G:C basepair in codon 12 or 13. However, in mice treated similarly with 6-NOC, the 1,2-DHD-6-AC-derived dG adduct accounted for 10% of the lung DNA adducts, and the N-hydroxy-6-AC-derived adduct accounted for 71% of the remaining adducts, which had the K-*ras* mutations at G:C (Li et al., 1994). Li et al. (1994) proposed that the tumor K-*ras* mutational spectra found in the lung tumors induced by 6-NC and its metabolites were due to the DNA damages induced by these compounds and not by expansion of pre-existing mutations.

Studies [discussed in Sections 5 and 6.1] have also identified DNA adducts of 6-NC metabolites in cells of target organs when exposed *in vitro:* human bronchial cells (Delclos et al., 1989) and rat hepatocytes (Delclos et al., 1987b, 1989). A study by Manjanatha et al. (1993) demonstrated that 6-NC-derived adducts were mutagenic in the *hprt* gene of CHO cells; 82% of the identified mutations in the *hprt* gene occurred at adenine-thymidine base pairs.

El Bayoumy et al. (1982; cited by IARC, 1989) found that 6-NC induced skin tumors, mainly papillomas, in a dermal initiation-promotion study in which 6-NC was used as the initiator, followed by promotion with the phorbol ester; and in a recent study conducted by Ogawa et al. (1996), 6-NC caused lung and forestomach tumors following i.p. injection to transgenic mice carrying a human hybrid c-Ha-*ras* gene. 6-Nitrochrysene is genotoxic in several assays in bacteria and mammalian cells, and metabolic pathways leading to mutagenic and clastogenic metabolites, and DNA adducts, have been described (IARC, 1989). Thus, it is likely that one or more genotoxic events are steps in the induction of tumors by 6-NC.

6.4 Structure-Activity Relationships

Summary: It has been reported that the mutagenicities of various nitroarenes were bilinearly related to the hydrophobicity of the compounds, with an optimal hydrophobicity constant (log P) of 5.44. An increase in the mutagenicity of nitropyrenes towards *S. typhimurium* as the number of nitro groups per compound increased was also found. Another study showed that a linear relationship existed between the first half-wave potential ($E_{1/2}$) and the logarithms of the mutagenicities of various nitroarenes towards *S. typhimurium*. Computer Automated Structure Evaluation (CASE) found two activating and 2 deactivating structures that were reported to be involved in the mediation of nitroarene mutagenicity in *S. typhimurium* (see Figure 6-2).

The mutagenic and carcinogenic potentials of nitroarene analogues vary. Some analogues are mutagenic and genotoxic in many systems, while other analogues are only mutagenic in some systems or are not mutagenic at all (for review see Klopman and Rosenkranz, 1984). Furthermore, some analogues are carcinogenic in rodents, while other analogues are not (Rosenkranz, 1987).

Several studies have been performed that identify structure activity relationships among the nitroarenes. For example, Mermelstein et al. (1982) reported that there was an increase in the mutagenicity of nitropyrenes (exogenous metabolic activation not mentioned) towards *S. typhimurium* strains TA98 and TA98NR as the number of nitro groups per compound increased. Mutagenicity towards these 2 strains increased in the order: 1-NP < 1,3-DNP < 1,6-DNP < 1,8-DNP. However, with further addition of nitro groups, the mutagenic activity of nitropyrenes decreased. After reaching a maximum with 1,8-DNP, mutagenicity declined for 1,3,6-trinitro- and 1,3,6,8-tetranitropyrene. In other strains of *S. typhimurium* (TA1537 and TA1538), the mutagenicity of 1-NP was 2 to 3 orders of magnitude lower than the mutagenicities of di-, tri-,

and tetranitropyrenes, but within the di-, tri-, and tetranitropyrenes there was no apparent pattern for increasing mutagenicity.

Klopman et al. (1984) reported that a linear relationship existed between the first half-wave potential ($E_{1/2}$) and the logarithms of the mutagenicities of various nitroarenes (including 1-NP, 1,6-DNP, and 1,8-DNP) towards *S. typhimurium* strains TA98 and TA1538. The lower the $E_{1/2}$, the more readily the nitroarene was nitroreduced. It was not stated whether exogenous metabolic activation was used. Since a linear relationship was also found to exist between $E_{1/2}$ and the calculated energies of the lowest unoccupied molecular orbital (LUMO), the authors suggested that the mutagenicities of nitroarenes could be predicted from their calculated LUMO energies. Debnath et al. (1992) reported that the mutagenic activity of aromatic and heteroaromatic nitro compounds (including 1-NP, 4-NP, 1,6-DNP, 1,8-DNP, and 6-NC) towards *S. typhimurium* strain TA100, without exogenous metabolic activation, was also linearly related to the LUMO energies of the compounds. Debnath et al. (1992) also reported that the mutagenicities of various nitroarenes were bilinearly related to the hydrophobicity of the compounds, with an optimal hydrophobicity constant (log P) of 5.44.

Klopman and Rosenkranz (1984) used the CASE program to predict the mutagenicity (without exogenous metabolic activation) of 53 nitroarenes (including 1-NP, 1,6-DNP, 1,8-DNP, and 6-NC) towards *S. typhimurium* strain TA98. Two activating and 2 deactivating structures were reported to be involved in the mediation of nitroarene mutagenicity (see Figure 6-2).

6.5 Cell Proliferation

Full details of the cell proliferation induced by 6-NC are presented in Table 6-2.

Summary: Hepatic nodular hyperplasia was detected in 1/26 newborn male mice treated with 6-NC (7.7 μg; 0.028 μmol; total dose) intraperitoneally. Similarly treated females and vehicle controls did not develop hepatic nodular hyperplasia. Another study conducted by the same group showed that the combined incidence (male and female) of hepatic nodular hyperplasia was significantly increased in newborn mice treated with 6-NC (38.5 μg; 0.14 μmol; total dose) intraperitoneally as compared to vehicle controls. However, when a higher dose of 6-NC (189 μg; 0.69 μmol; total dose) was similarly administered, the incidence of hepatic nodular hyperplasia did not differ significantly between 6-NC-treated mice (males or females) and appropriate vehicle controls.

In a study of the role of the human hybrid c-Ha-*ras* gene in the mediation of tumor induction by 6-NC, the incidence of pulmonary hyperplasia did not differ significantly between transgenic (containing the human hybrid c-Ha-*ras* gene) and non-transgenic 6-NC-treated mice.

Hepatic nodular hyperplasia was detected in 1/26 newborn male Swiss-Webster BLU:Ha mice treated with a 7.7 μg (0.028 μmol) total dose of 6-NC (1/7 of total dose administered at birth, 2/7 on day 8, 4/7 on day 15) intraperitoneally. Similarly treated females and vehicle controls did not develop hepatic nodular hyperplasia. None of the mice (6-NC-treated or control) developed liver tumors (Busby et al., 1989).

The combined incidence (male and female) of hepatic nodular hyperplasia was significantly increased in newborn Swiss-Webster BLU:Ha mice treated with a 38.5 μg (0.14 μmol) total dose of 6-NC (1/7 of total dose administered at birth, 2/7 on day 8, 4/7 on day 15) intraperitoneally as compared to vehicle controls. However, when a higher dose (189 μg; 0.69

μmol) of 6-NC was similarly administered, the incidence of hepatic nodular hyperplasia did not differ significantly between 6-NC-treated mice (males or females) and appropriate vehicle controls (Busby et al., 1985).

In a study of the role of the human hybrid c-Ha-*ras* gene in the mediation of tumor induction by 6-NC, 9-week-old male and female non-transgenic and transgenic C57BL/6xBALB/c mice were treated with 2.2 μmol 6-NC intraperitoneally once every 2 weeks for 4 weeks (for a total of 3 injections). Four of nine 6-NC-treated transgenic males, 4/7 6-NC-treated transgenic females, 5/13 6-NC-treated non-transgenic males, 2/10 6-NC-treated non-transgenic females, 0/4 transgenic male controls, 1/3 transgenic female controls, 1/4 non-transgenic male controls, and 0/4 female non-transgenic controls developed pulmonary hyperplasia. The incidence of pulmonary hyperplasia did not differ significantly between transgenic and non-transgenic 6-NC-treated mice. Statistical analyses were not done to determine whether the incidence of pulmonary hyperplasia differed between 6-NC-treated and control mice (Ogawa et al., 1996).

6.6 6-Nitrochrysene with Other Treatments

The incidence of TPA-induced skin tumors (mainly papillomas) was significantly increased in 50- to 55-day-old female CD-1 Charles River mice treated with 0.1 mg (0.4 μmol) 6-NC on shaved dorsal skin, every other day for 20 days, followed ten days later with application of 2.5 μg *O*-tetradecanoylphorbol 13-acetate in 0.1 mL acetone, 3 times per week for 25 weeks (El-Bayoumy et al., 1982; cited by IARC, 1989).

Table 6-1. 6-Nitrochrysene Metabolite and DNA Adduct Identification

Name (No.[1])	In Vivo	In Vitro	Bacteria	Reaction/Enzymes	Comments
Parent Compound					
6-Nitrochrysene; 6-NC **(I)**	1- and 8-day-old BLU:HA mice (Delclos et al., 1988a). Conventional and germfree Balb/c mice (Delclos et al., 1990a).	Human bronchus explants (Delclos et al., 1989). Human hepatic and pulmonary microsomes (Chae et al., 1993). Mouse liver S9 (Delclos et al., 1988b). Human, rat, and mouse intestinal microflora (Manning et al., 1988).	*S. typhimurium* TA100 incubated with liver S9 derived from Aroclor 1254-induced rats (El-Bayoumy et al., 1989).	Cytochromes P-450 IA1, IA2, and 3A4 were listed as isozymes involved in the metabolism of I (Chae et al., 1993; Gonzalez et al., 1994). Nitroreduction and a combination of nitroreduction and ring oxidation (Li et al., 1993). Aryl hydrocarbon hydroxylase (AHH) activity was elevated in all examined organs (liver, lung, colon) of mice and rats i.p. injected with 6-NC (Imaida et al., 1992).	Formation of VI and III was greater in 1-day-old mice (Delclos et al., 1988a). Human hepatic and pulmonary microsomes produced qualitatively similar metabolites; however, quantitative differences were observed. Human lung and liver are capable of metabolizing I to known potent carcinogenic metabolites via ring oxidation and nitroreduction (Chae et al., 1993). Germfree mice excreted VI in feces at ~25% of that excreted by conventional mice treated similarly (Delclos et al., 1990a). Nitroreduction varied among human, rat, and mouse intestinal microflora, with human intestinal microflora metabolizing I to the greatest extent (Manning et al., 1988). Imaida et al. (1992) found that AHH levels in all organs examined were elevated by 6-NC treatment, with the induction rate in mouse lung being the highest. AHH is widely regarded as an important enzyme determining tumorigenesis in specific organs, especially for PAHs (Imaida et al., 1992).
Metabolites					
6-Nitrosochrysene; 6-NOC (II)		Human intestinal microflora (Manning et al., 1988). CHO cells (Delclos et al., 1993).		Nitroreduction (Delclos et al., 1993).	II nitroreduced to IV (Delclos et al., 1993).

Table 6-1. 6-Nitrochrysene Metabolite and DNA Adduct Identification (Continued)

Name (No.[1])	In Vivo	In Vitro	Bacteria	Reaction/Enzymes	Comments
***trans*-1,2-Dihydrodiol-6-nitro-chrysene; 1,2-DHD-6-NC (III)**	1- and 8-day-old BLU:HA mice (Delclos et al., 1988a).	Human hepatic and pulmonary microsomes (Chae et al., 1993). Mouse liver S9 (Delclos et al., 1988). Rat liver exogenous metabolic system (El-Bayoumy and Hecht, 1984).	*S. typhimurium* TA100 incubated with liver S9 derived from Aroclor 1254-induced rats (El-Bayoumy et al., 1989).	Ring hydroxylation	Following incubation of *S. typhimurium* TA100 with liver S9, 1,2-dihydroxy-6-nitrochrysene was identified as a minor metabolite (El-Bayoumy et al., 1989).
***trans*-9,10-Dihydrodiol-6-nitrochrysene; 9,10-DHD-6-NC**	1- and 8-day-old BLU:HA mice (Delclos et al., 1988a).	Human hepatic and pulmonary microsomes (Chae et al., 1993). Mouse liver S9 (Delclos et al., 1988b).	*S. typhimurium* TA100 incubated with liver S9 derived from Aroclor 1254-induced rats (El-Bayoumy et al., 1989).	Ring hydroxylation	
***N*-Hydroxy-6-aminochrysene; *N*-OH-6-AC (IV)**	Conventional and germfree Balb/c mice (Delclos et al., 1990a).	CHO cells (Delclos et al., 1993).		Nitroreduction (Delclos et al., 1993).	IV incubated with calf thymus DNA produced DNA adducts VIII, IX, and X (Delclos et al., 1987a). See comments for VII.
***trans*-1,2-Dihydrodiol-6-aminochrysene; 1,2-DHD-6-AC (V)**	1- and 8-day-old BLU:HA mice (Delclos et al., 1988a). Conventional and germfree Balb/c mice (Delclos et al., 1990a).	Human hepatic and pulmonary microsomes (Chae et al., 1993). Mouse liver S9 (Delclos et al., 1988b). CHO cells (Li et al., 1993).		Ring hydroxylation and nitroreduction (Li et al., 1993). Cytochrome P-450$_{BNE-B}$ (Delclos et al., 1988a).	IV and V showed preferential binding to liver, while only V showed a preference for lung (Delclos et al., 1990a). V is the ultimate carcinogenic species formed from both 6-AC and 6-NC (Delclos et al., 1988b).

Table 6-1. 6-Nitrochrysene Metabolite and DNA Adduct Identification (Continued)

Name (No.[1])	In Vivo	In Vitro	Bacteria	Reaction/Enzymes	Comments
6-Aminochrysene; 6-AC (VI)	1- and 8-day-old BLU:HA mice (Delclos et al., 1988a). Feces of conventional and germfree Balb/c mice (Delclos et al., 1990a). Feces of Sprague-Dawley rats (Delclos et al., 1993).	Human hepatic and pulmonary microsomes (Chae et al., 1993). Human intestinal microflora (Manning et al., 1988). Mouse liver S9 (Delclos et al., 1988b). Rat liver exogenous metabolic system (El-Bayoumy and Hecht, 1984).	*S. typhimurium* TA100 incubated with liver S9 derived from Aroclor 1254-induced rats (El-Bayoumy et al., 1989).	Nitroreduction. Cytochromes P-450 2B6 and 3A4 were listed as isozymes involved in the metabolism of VI (Gonzalez et al., 1994).	
Chrysene-5,6-quinone	Feces of Sprague-Dawley rats (Delclos et al., 1993).	Human hepatic and pulmonary microsomes (Chae et al., 1993).			
1,2-Dihydrodiol-6-aminochrysene-3,4-epoxide; 1,2-DHD-6-AC-3,4-epoxide (VII)	Preweanling mice (Delclos et al., 1988a).	CHO cells incubated with I and VI and their metabolites (Delclos and Heflich, 1992).			
***N*-Formyl-6-aminochrysene; 6-FAC**		Human intestinal microflora (Manning et al., 1988).			The ratio of VI to 6-FAC to II 48 h after introduction of [^{3}H]I into the culture vessel was 93.4 : 6.3 : 0.3, respectively (Manning et al., 1988).
DNA Adducts					
5-(Deoxyguanosin-N^2-yl)-6-aminochrysene; 5-(dG-N^2-yl)-6-AC (VIII)	Rats i.p. injected with I (Chae et al., 1996). Mouse liver (Delclos et al., 1990a)	Human AHH-1 cells (Morris et al., 1994). IV incubated with calf thymus DNA (Delclos et al., 1987a). CHO cells (Mugimane et al., 1993; Delclos et al., 1993).		20 to 30 nmol bound/mg DNA/30 min (Delclos et al., 1987a).	Adducts VIII and IX derived from V (Delclos et al., 1993, cited by Morris et al., 1994). In agreement with Chae et al. (1996). [Metabolic activation system not included in incubations]. VIII, IX, and X accounted for 32%, 28%, and 22%, respectively, of the total DNA adducts formed (Delclos et al., 1987a).

Table 6-1. 6-Nitrochrysene Metabolite and DNA Adduct Identification (Continued)

Name (No.[1])	In Vivo	In Vitro	Bacteria	Reaction/Enzymes	Comments
***N*-(Deoxyguanosin-8-yl)-6-aminochrysene; *N*-(dG-8-yl)-6-AC (IX)**	Rats i.p. injected with I (Chae et al., 1996). Mouse liver (Delclos et al., 1990a)	Human AHH-1 cells (Morris et al., 1994). IV incubated with calf thymus DNA; rat hepatocytes treated with I or VI (Delclos et al., 1987a). CHO cells (Mugimane et al., 1993; Delclos et al., 1993).		20 to 30 nmol bound/mg DNA/30 min (Delclos et al., 1987a).	Adducts IX and XI derived from IV and detected as minor adducts (Delclos et al., 1987a, 1990; Chae et al., 1996). [Metabolic activation system not included in incubations (Delclos et al., 1987a].
***N*-(Deoxyadenosine-8-yl)-6-aminochrysene; *N*-(DA-8-yl)-6-AC (X)**		IV incubated with calf thymus DNA; rat hepatocytes treated with I or VI (Delclos et al., 1987a). CHO cells (Mugimane et al., 1993; Delclos et al., 1993).		Twenty to 30 nmol bound/mg DNA/30 min (Delclos et al., 1987a).	[Metabolic activation system not included in incubations].
***N*-(Deoxyinosin-8-yl)-6-aminochrysene; *N*-(DI-8-yl)-6-aminochrysene XI**	Rats i.p. injected with I (Chae et al., 1996). Mouse liver (Delclos et al., 1990a)	Human AHH-1 cells (Morris et al., 1994). IV incubated with calf thymus DNA; rat hepatocytes treated with I or VI (Delclos et al., 1987a). CHO cells (Mugimane et al., 1993).		Postulated to be formed from spontaneous oxidation of the corresponding deoxyadenosine adduct (X) prior to or during DNA isolation and adduct preparation. 20 to 30 nmol bound/mg DNA/30 min (Delclos et al., 1987a; Mugimane et al., 1993). DNA isolated from rat hepatocytes contained up to 12 pmol adducts/mg DNA [4 adducts /10^6 nucleotides] (Delclos et al. 1987a).	[Metabolic activation system not included in incubations (Delclos et al., 1987a]. Possible artifact (Delclos et al., 1987a).

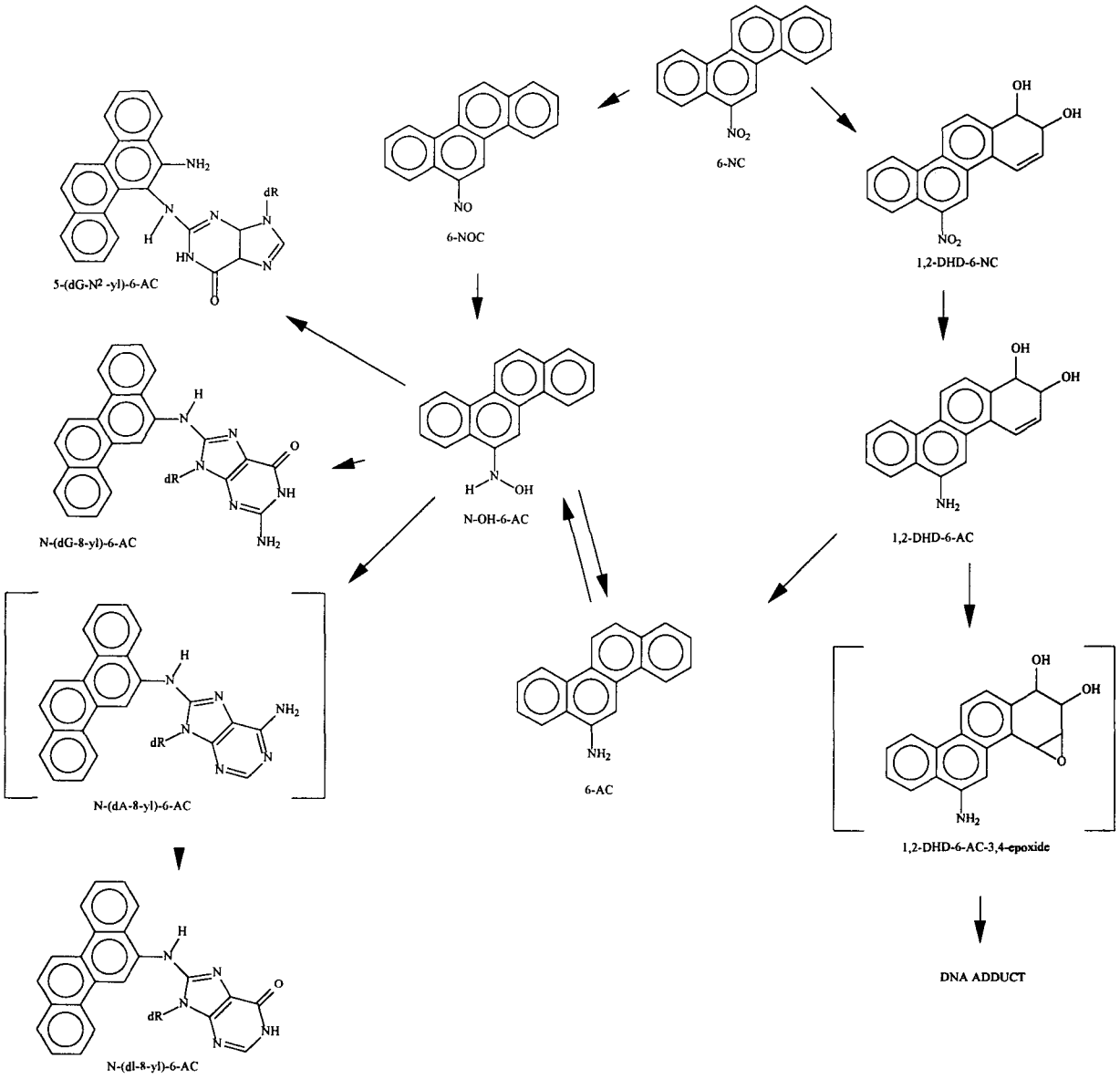
Table 6-2. Cell Proliferation Induced by 6-Nitrochrysene

Age, Strain, Species	No. and Sex Exposed	Controls	Chemical Form and Purity	Dose	Duration of Exposure	Results/Comments	Reference
Mice - *Intraperitoneal Injection*							
newborn Swiss-Webster BLU:Ha mouse	effective number: 26M, 22F	effective number: 91M, 101F (DMSO alone)	6-NC, > 99.9% pure	7.7 µg (0.03 µmol) total dose in DMSO, injected i.p. 1/7 of total dose (1.1 µg; 0.0040 µmol) administered at birth, 2/7 (2.2 µg; 0.0080 µmol) on day 8, 4/7 (4.4 µg; 0.016 µmol) on day 15	15 days	Mice were killed when they were 26 weeks old. Histopathological examination of lung and abnormal tissue was performed. **All Tissues:** Negative There was no increase in proliferative activity in the examined tissues of 6-NC-treated mice.	Busby et al. (1989)
newborn Swiss-Webster BLU:Ha mouse	22M, 29F (low dose) 23M, 21F (high dose)	22M, 15F (DMSO alone)	6-NC, purity not specified	38.5 µg (140 nmol) or 189 µg (690 nmol) total dose in DMSO, injected i.p. 1/7 of total dose (5.5 or 27 µg; 0.020 or 0.099 µmol) administered at birth, 2/7 (11 or 54 µg; 0.040 or 0.20 µmol) on day 8, 4/7 (22 or 108 µg; 0.080 or 0.39 µmol) on day 15	15 days	Mice were killed when they were 26 weeks old. Lungs and any abnormal tissues or organs were examined histologically. Statistical analyses of incidence were performed using the method of Peto et al. (1980; cited by Busby et al., 1985), without correction for intercurrent mortality. **Liver:** Positive (for proliferative activity, as indicated by presence of hyperplasia; low-dose group only) The incidence of hepatic nodular hyperplasia in mice treated with the low dose was significantly increased when males and females were pooled (6/22 males and 1/29 females vs. 0/22 male controls and 0/15 female controls; $p < 0.001$). The incidence of hepatic nodular hyperplasia in high-dose mice was not significantly increased.	Busby et al. (1985)

Table 6-2. Cell Proliferation Induced by 6-Nitrochrysene (Continued)

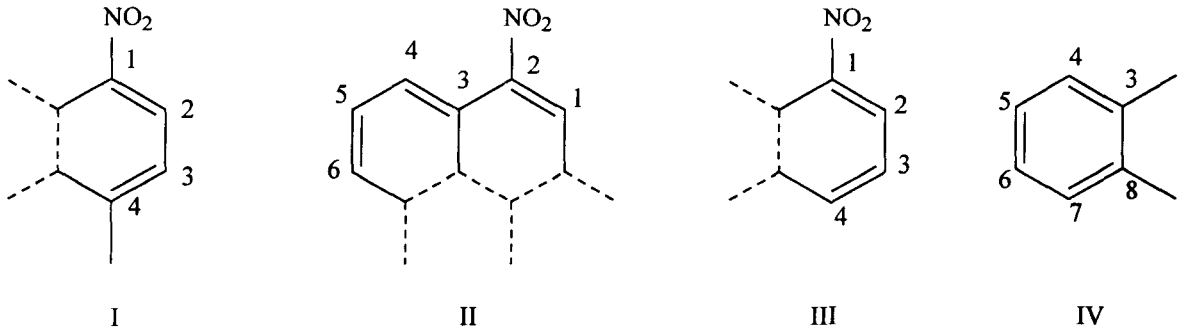
Age, Strain, Species	No. and Sex Exposed	Controls	Chemical Form and Purity	Dose	Duration of Exposure	Results/Comments	Reference
9-wk-old non-transgenic C57BL/6×BALB/c mouse and transgenic C57BL/6×BALB/c mouse, containing the human hybrid c-Ha-*ras* gene	number of 6-NC-exposed mice not specified, but 22M and 19F non-transgenic mice, and 19M and 15F transgenic mice were included in the study	number of DMSO controls not specified, but 22M and 19F non-transgenic mice, and 19M and 15F transgenic mice were included in the study	6-NC, purity not specified	2.2 μmol in DMSO, injected i.p., 3 times, at 2-week intervals	4 wk	Surviving animals were killed at the end of week 25. All organs were examined. **Lungs:** Negative The incidence of pulmonary hyperplasia was increased in 6-NC-treated animals (4/9 transgenic males and 4/7 treated transgenic females vs. 0/4 transgenic male controls and 1/3 transgenic female controls; 5/13 non-transgenic males and 2/10 treated non-transgenic females vs. 1/4 non-transgenic male controls and 0/4 non-transgenic female controls). However, statistical analyses to determine significance were not performed. The incidence of pulmonary hyperplasia did not differ significantly between transgenic and non-transgenic animals treated with 6-NC.	Ogawa et al. (1996)

Figure 6-1. Metabolic Activation of 6-Nitrochrysene



Source: Li et al. (1994)

Figure 6-2. Fragments Responsible for the Mutagenicity of Nitroarenes



I II III IV

Fragments I and II are required for activity while fragments III and IV are deactivating. I differs from III in that C-4 is not bonded to a hydrogen (Klopman and Rosenkranz, 1984).

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APPENDIX A

DESCRIPTION OF ONLINE SEARCHES FOR THE NITROARENES

DESCRIPTION OF ONLINE SEARCHES FOR THE NITROARENES (IARC Monograph in Vol. 46, 1989)

Online searching was done by the technical support contractor in TOXLINE January 30, 1996, using the CASRNs of the title compounds and *o*-nitroanisole and specifying publications after 1988. IARC (1989) was to be relied on for identification of pertinent earlier references. The 1240 records in TOXLINE were reduced by combining with the controlled vocabulary terms for metabolism and neoplasms and with the free-text truncated terms carcinogen? or mechanis? or toxicokinetic? or metab? From the 418 resulting records, the contractor selected approximately 160 for acquisition. Of the approximately 100 citations related to biological activity independently selected by the primary reviewer from NIEHS Review Group 1, 20 were identified as abstracts for which full publications were available; 73 had also been selected by the contractor. Thus, the primary reviewer selected 7 additional references that had not been identified as potentially useful by the contractor.

An exhaustive search of other pertinent toxicology databases was not attempted for the nitroarenes. A high degree of redundancy had been noted between TOXLINE and the databases CANCERLIT, EMBASE (Excerpta Medica), MEDLINE, and NIOSHTIC (Occupational Safety and Health). No special attempt was made to find toxicity information about metabolites and other structural analogues in the search strategy.

The contractor also searched CSCHEM and CSCORP for U.S. suppliers (Chem Sources databases); EMIC; EMICBACK; HSDB; IRIS; TSCATS (Toxic Substances Control Act Test Submissions); the Chemical Information System's databases SANSS (the Structure and Nomenclature Search System), ISHOW (for physical-chemical properties), and REGMAT (May 1993 version; this regulatory information database with broad coverage of EPA regulations is no longer available); Chemical Abstracts Service's (CAS) CA and Registry Files for metabolism studies (152 records) and metabolite identification; CAS File CHEMLIST for TSCA and SARA updates in 1996; and CA File sections 59 (Air Pollution and Industrial Hygiene), 60 (Waste Disposal and Treatment), and 61 (Water) for environmental exposure information. For current awareness, the contractor monitored Current Contents on Diskette[7] Life Sciences 1200 [journals] edition. Older literature that needed to be examined was identified from the reviews and original articles as they were acquired.

APPENDIX B

LISTING OF GAP TEST CODES IN ALPHABETICAL ORDER

LISTING OF GAP TEST CODES IN ALPHABETICAL ORDER

Test Code	Definition
ACC	Allium cepa, chromosomal aberrations
AIA	Aneuploidy, animal cells in vitro
AIH	Aneuploidy, human cells in vitro
ANF	Aspergillus nidulans, forward mutation
ANG	Aspergillus nidulans, genetic crossing-over
ANN	Aspergillus nidulans, aneuploidy
ANR	Aspergillus nidulans, reverse mutation
ASM	Arabidopsis species, mutation
AVA	Aneuploidy, animal cells in vivo
AVH	Aneuploidy, human cells in vivo
BFA	Body fluids from animals, microbial mutagenicity
BFH	Body fluids from humans, microbial mutagenicity
BHD	Binding (covalent) to DNA, human cells in vivo
BHP	Binding (covalent) to RNA or protein, human cells in vivo
BID	Binding (covalent) to DNA in vitro
BIP	Binding (covalent) to RNA or protein in vitro
BPF	Bacteriophage, forward mutation
BPR	Bacteriophage, reverse mutation
BRD	Other DNA repair-deficient bacteria, differential toxicity
BSD	Bacillus subtilis rec strains, differential toxicity
BSM	Bacillus subtilis multi-gene test
BVD	Binding (covalent) to DNA, animal cells in vivo
BVP	Binding (covalent) to RNA or protein, animal cells in vivo
CBA	Chromosomal aberrations, animal bone-marrow cells in vivo
CBH	Chromosomal aberrations, human bone-marrow cells in vivo
CCC	Chromosomal aberrations, spermatocytes treated in vivo and cytes obs.
CGC	Chromosomal aberrations, spermatogonia treated in vivo and cytes obs.
CGG	Chromosomal aberrations, spermatogonia treated in vivo and gonia obs.
CHF	Chromosomal aberrations, human fibroblasts in vitro
CHL	Chromosomal aberrations, human lymphocyte in vitro
CHT	Chromosomal aberrations, transformed human cells in vitro
CIA	Chromosomal aberrations, other animal cells in vitro
CIC	Chromosomal aberrations, Chinese hamster cells in vitro
CIH	Chromosomal aberrations, other human cells in vitro
CIM	Chromosomal aberrations, mouse cells in vitro
CIR	Chromosomal aberrations, rat cells in vitro
CIS	Chromosomal aberrations, Syrian hamster cells in vitro
CIT	Chromosomal aberrations, transformed animal cells in vitro
CLA	Chromosomal aberrations, animal leukocytes in vivo
CLH	Chromosomal aberrations, human lymphocytes in vivo

Test Code	Definition
COE	Chromosomal aberrations, oocytes or embryos treated in vivo
CVA	Chromosomal aberrations, other animal cells in vivo
CVH	Chromosomal aberrations, other human cells in vivo
DIA	DNA strand breaks, cross-links or rel. damage, animal cells in vitro
DIH	DNA strand breaks, cross-links or rel. damage, human cells in vitro
DLM	Dominant lethal test, mice
DLR	Dominant lethal test, rats
DMC	Drosophila melanogaster, chromosomal aberrations
DMG	Drosophila melanogaster, genetic crossing-over or recombination
DMH	Drosophila melanogaster, heritable translocation test
DML	Drosophila melanogaster, dominant lethal test
DMM	Drosophila melanogaster, somatic mutation (and recombination)
DMN	Drosophila melanogaster, aneuploidy
DMX	Drosophila melanogaster, sex-linked recessive lethal mutation
DVA	DNA strand breaks, cross-links or rel. damage, animal cells in vivo
DVH	DNA strand breaks, cross-links or rel. damage, human cells in vivo
ECB	Escherichia coli (or E. coli DNA), strand breaks, cross-links or repair
ECD	Escherichia coli pol A/W3110-P3478, diff. toxicity (spot test)
ECF	Escherichia coli (excluding strain K12), forward mutation
ECK	Escherichia coli K12, forward or reverse mutation
ECL	Escherichia coli pol A/W3110-P3478, diff. toxicity (liquid susp. test)
ECR	Escherichia coli, miscellaneous strains, reverse mutation
ECW	Escherichia coli WP2 uvrA, reverse mutation
EC2	Escherichia coli WP2, reverse mutation
ERD	Escherichia coli rec strains, differential toxicity
FSC	Fish, chromosomal aberrations
FSI	Fish, micronuclei
FSM	Fish, mutation
FSS	Fish, sister chromatid exchange
FSU	Fish, unscheduled DNA synthesis
GCL	Gene mutation, Chinese hamster lung cells exclusive of V79 in vitro
GCO	Gene mutation, Chinese hamster ovary cells in vitro
GHT	Gene mutation, transformed human cells in vivo
GIA	Gene mutation, other animal cells in vitro
GIH	Gene mutation, human cells in vitro
GML	Gene mutation, mouse lymphoma cells exclusive of L5178Y in vitro
GVA	Gene mutation, animal cells in vivo
G5T	Gene mutation, mouse lymphoma L5178Y cells in vitro, TK locus
G51	Gene mutation, mouse lymphoma L5178Y cells in vitro, all other loci
G9H	Gene mutation, Chinese hamster lung V-79 cells in vitro, HPRT locus
G9O	Gene mutation, Chinese hamster lung V-79 cells in vitro, ouabain resistance
HIM	Haemophilus influenzae, mutation
HMA	Host mediated assay, animal cells in animal hosts

Test Code	Definition
HMH	Host mediated assay, human cells in animal hosts
HMM	Host mediated assay, microbial cells in animal hosts
HSC	Hordeum species, chromosomal aberrations
HSM	Hordeum species, mutation
ICH	Inhibition of intercellular communication, human cells in vitro
ICR	Inhibition of intercellular communication, rodent cells in vitro
KPF	Klebsiella pneumonia, forward mutation
MAF	Micrococcus aureus, forward mutation
MHT	Mouse heritable translocation test
MIA	Micronucleus test, animal cells in vitro
MIH	Micronucleus test, human cells in vitro
MST	Mouse spot test
MVA	Micronucleus test, other animals in vivo
MVC	Micronucleus test, hamsters in vivo
MVH	Micronucleus test, human cells in vivo
MVM	Micronucleus test, mice in vivo
MVR	Micronucleus test, rats in vivo
NCF	Neurospora crassa, forward mutation
NCN	Neurospora crassa, aneuploidy
NCR	Neurospora crassa, reverse mutation
PLC	Plants (other), chromosomal aberrations
PLI	Plants (other), micronuclei
PLM	Plants (other), mutation
PLS	Plants (other), sister chromatid exchanges
PLU	Plants, unscheduled DNA synthesis
PRB	Prophage, induction, SOS repair, DNA strand breaks, or cross-links
PSC	Paramecium species, chromosomal aberrations
PSM	Paramecium species, mutation
RIA	DNA repair exclusive of UDS, animal cells in vitro
RIH	DNA repair exclusive of UDS, human cells in vitro
RVA	DNA repair exclusive of UDS, animal cells in vivo
SAD	Salmonella typhimurium, DNA repair-deficient strains, differential toxicity
SAF	Salmonella typhimurium, forward mutation
SAL	Salmonella typhimurium, all strains, reverse mutation
SAS	Salmonella typhimurium (other misc. strains), reverse mutation
SA0	Salmonella typhimurium TA100, reverse mutation
SA1	Salmonella typhimurium TA97, reverse mutation
SA2	Salmonella typhimurium TA102, reverse mutation
SA3	Salmonella typhimurium TA1530, reverse mutation
SA4	Salmonella typhimurium TA104, reverse mutation
SA5	Salmonella typhimurium TA1535, reverse mutation
SA7	Salmonella typhimurium TA1537, reverse mutation
SA8	Salmonella typhimurium TA1538, reverse mutation

Test Code	Definition
SA9	Salmonella typhimurium TA98, reverse mutation
SCF	Saccharomyces cerevisiae, forward mutation
SCG	Saccharomyces cerevisiae, gene conversion
SCH	Saccharomyces cerevisiae, homozygosis by recombination or gene conversion
SCN	Saccharomyces cerevisiae, aneuploidy
SCR	Saccharomyces cerevisiae, reverse mutation
SGR	Streptomyces griseoflavus, reverse mutation
SHF	Sister chromatid exchange, human fibroblasts in vitro
SHL	Sister chromatid exchange, human lymphocytes in vitro
SHT	Sister chromatid exchange, transformed human cells in vitro
SIA	Sister chromatid exchange, other animal cells in vitro
SIC	Sister chromatid exchange, Chinese hamster cells in vitro
SIH	Sister chromatid exchange, other human cells in vitro
SIM	Sister chromatid exchange, mouse cells in vitro
SIR	Sister chromatid exchange, rat cells in vitro
SIS	Sister chromatid exchange, Syrian hamster cells in vitro
SIT	Sister chromatid exchange, transformed cells in vitro
SLH	Sister chromatid exchange, human lymphocytes in vivo
SLO	Mouse specific locus test, other stages
SLP	Mouse specific locus test, postspermatogonia
SPF	Sperm morphology, F1 mouse
SPH	Sperm morphology, human
SPM	Sperm morphology, mouse
SPR	Sperm morphology, rat
SPS	Sperm morphology, sheep
SSB	Saccharomyces species, DNA breaks, cross-links or related damage
SSD	Saccharomyces cerevisiae, DNA repair-deficient strains, diff. toxicity
STF	Streptomyces coelicolor, forward mutation
STR	Streptomyces coelicolor, reverse mutation
SVA	Sister chromatid exchange, animal cells in vivo
SVH	Sister chromatid exchange, other human cells in vivo
SZD	Schizosaccharomyces pombe, DNA repair-deficient strains, diff. toxicity
SZF	Schizosaccharomyces pombe, forward mutation
SZG	Schizosaccharomyces pombe, gene conversion
SZR	Schizosaccharomyces pombe, reverse mutation
T7R	Cell transformation, SA7/rat cells
T7S	Cell transformation, SA7/Syrian hamster embryo cells
TBM	Cell transformation, BALB/C3T3 mouse cells
TCL	Cell transformation, other established cell lines
TCM	Cell transformation, C3H10T1/2 mouse cells
TCS	Cell transformation, Syrian hamster embryo cells, clonal assay
TEV	Cell transformation, other viral enhancement systems
TFS	Cell transformation, Syrian hamster embryo cells, focus assay

Test Code	Definition
TIH	Cell transformation, human cells in vitro
TPM	Cell transformation, mouse prostate cells
TRR	Cell transformation, RLV/Fischer rat embryo cells
TSC	Tradescantia species, chromosomal aberrations
TSI	Tradescantia species, micronuclei
TSM	Tradescantia species, mutation
TVI	Cell transformation, treated in vivo, scored in vitro
UBH	Unscheduled DNA synthesis, human bone-marrow cells in vivo
UHF	Unscheduled DNA synthesis, human fibroblasts in vitro
UHL	Unscheduled DNA synthesis, human lymphocytes in vitro
UHT	Unscheduled DNA synthesis, transformed human cells in vitro
UIA	Unscheduled DNA synthesis, other animal cells in vitro
UIH	Unscheduled DNA synthesis, other human cells in vitro
UPR	Unscheduled DNA synthesis, rat hepatocytes in vivo
URP	Unscheduled DNA synthesis, rat primary hepatocytes
UVA	Unscheduled DNA synthesis, other animal cells in vivo
UVC	Unscheduled DNA synthesis, hamster cells in vivo
UVH	Unscheduled DNA synthesis, other human cells in vivo
UVM	Unscheduled DNA synthesis, mouse cells in vivo
UVR	Unscheduled DNA synthesis, rat cells (other than hepatocytes) in vivo
VFC	Vicia faba, chromosomal aberrations
VFS	Vicia faba, sister chromatid exchange